The Journal of
Clinical Endocrinology and Metabolism

VOLUME 20 JUNE, 1960 NUMBER 6
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URINARY ESTROGEN EXCRETION IN NORMAL AND DIABETIC PREGNANCY*†

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ABSTRACT

Urinary levels of estriol, estrone and estradiol-17β (estradiol) increase during the last 20 weeks of pregnancy in a qualitatively similar fashion in both the normal and diabetic states. The relative amounts of these estrogens found in the urine suggest that they arise from the same source in both groups according to the transformation, estradiol$\rightleftharpoons$estrone$\rightarrow$estriol. It also appears likely that there is an additional source of estriol in the two groups, particularly in late pregnancy. There is a tendency for estriol levels to be lower in diabetic than in nondiabetic pregnancy. Considerable variability in the relative amounts of these 3 estrogens from one pregnancy to another, both diabetic and normal, suggests the occurrence of a varying pattern of estrogen metabolism from one individual to another.

INTRODUCTION

COMPLICATIONS during pregnancy in patients with diabetes mellitus, particularly the high incidence of intra-uterine death, have been recognized for some time. Despite this, little precise information is available regarding the biochemical aspects of the problem. Abnormal levels of gonadotropins and pregnanediol have been reported in diabetic pregnancy (1) but the field of estrogen metabolism has been largely neglected. Rubin *et al.* (2), using a chemical method of assay, reported data on

Received December 4, 1959.

* Supported by grants from The Women's Auxiliary of the Montreal General Hospital and Ayerst, McKenna and Harrison Ltd.

† Some of the early work was under the supervision of the late Dr. W. S. Bauld.

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a few patients showing that urinary estrogen excretion may be normal in diabetic pregnancy uncomplicated by pre-eclamptic toxemia, but low when toxemia is present.

The present investigation was undertaken with a view to ascertaining whether or not diabetic pregnancy has associated with it any abnormality in the urinary estrogen pattern. To this end estriol, estrone and estradiol-17β (estradiol) were measured by an established chemical procedure (3). The biochemical aspects of the study are presented in this paper.

MATERIAL AND METHODS

Subjects

The control group consisted of 10 pregnant women exhibiting no clinical abnormalities. The diabetic group, none of whom exhibited pre-eclamptic toxemia, numbered 14 pregnant women with varying degrees of diabetes mellitus, all of whom were maintained with insulin. None of the subjects, normal or otherwise, was receiving any other type of medication. The two groups were studied between the twentieth week of pregnancy and delivery. Estrogen determinations were made weekly in the normal group, and from 3 to 15 estimations were carried out on each of the diabetic patients over the twenty-week period.

Urine collection

Urine was collected as 24-hour specimens in specially cleaned polyethylene bottles without preservative. Estrogen determinations were made as soon as possible thereafter. When delay was necessary, the urine was stored in the refrigerator at 4°C. until the assay could be performed.

Estrogen determination

Estriol, estrone and estradiol were measured chemically by the method described by Bauld (3), or a modification of this procedure (4). An aliquot of from 10 to 50 ml. of the 24-hour urine specimen was taken for analysis, depending upon the stage of pregnancy. Diabetic urines were diluted 1:10 with distilled water prior to acid hydrolysis in order to overcome the destructive effect of glucose on the urinary estrogens (4). When the larger urine aliquots were necessary, the original Bauld procedure was followed after hydrolysis. This involves partition of a phenolic extract between benzene and water, with subsequent partition chromatography of the benzene (estrone-estradiol) fraction and saponification of the aqueous (estriol) extract. The next stage consists of saponification of the separated estrone and estradiol, and partition chromatography of the estriol fraction. The 3 separated estrogens are then measured spectrophotometrically by the Kober reaction. All analyses were performed on duplicate urine aliquots. When small volumes of urine could be used (*e.g.*, 10 ml.), saponification of the estrone and estradiol fractions and chromatography of the estriol fraction were omitted (4, 5). The reliability of these chemical procedures as carried out in this laboratory has already been established for urines not containing glucose, as discussed elsewhere (5, 6).

To obtain information on recovery of the 3 estrogens in the presence of glucose, 10-ml. portions of 7 nonpregnancy human urines were subjected to acid hydrolysis after 1:10 and 1:25 dilution both with and without the addition of 2–4 per cent glucose to the original urine. Prior to hydrolysis, 25–50 μg. of estriol, 10.9 μg. of estrone and 10 μg. of es-

tradiol were added to each diluted aliquot. A total of 32 duplicate analyses was performed. Percentage recoveries of estriol (with standard deviations) at 1:10 dilution, with and without 2 per cent glucose, were 76 ± 3.4 and 79 ± 3.9, respectively. Corresponding values for the recovery of estrone were 87 ± 1.9 and 92 ± 2.1, and for estradiol 77 ± 3.1 and 83 ± 2.5. At 1:25 dilution there was no significant increase in recovery. Thus for estriol, with and without 2 per cent glucose, the percentages were 78 ± 1.0 and 79 ± 3.7. In 2 experiments in which 4 per cent glucose was added prior to 1:10 dilution, the mean recovery of added estriol was 75 per cent. In the insulin-controlled diabetic group, the highest level of urinary sugar recorded was 2 per cent.

Recovery of estriol and estradiol was somewhat lower than might have been expected from earlier work (5). The foregoing results, however, make it legitimate to compare levels of the 3 estrogens in diabetic and in nondiabetic pregnancy urines diluted 1:10 before acid hydrolysis. Moreover, since further dilution does not significantly influence recovery of added estrogen, it is likely that optimum levels of estriol, estrone and estradiol are measured at 1:10 dilution. It is accepted that the effect of acid hydrolysis on free estrogens in the presence of glucose is not necessarily the same as on urinary estrogen conjugates. Until these conjugates have been characterized, however, more precise information will not be available.

RESULTS

Normal pregnancy

The urinary levels of estriol, estrone and estradiol between the twentieth and fortieth weeks of pregnancy are shown in Figures 1, 2 and 3. Estrone and estradiol showed parallel rises, each increasing by about 165 per cent during the period studied. Estriol showed an even greater average increase of about 320 per cent during this time. These findings, both qualitatively and quantitatively, are in good general agreement with those reported by Brown (7). The average ratio of estrone to estradiol (referred to hereafter as E_1/E_2) calculated at 20, 25, 30, 35 and 40 weeks varied only slightly, lying in the range 3.2–4.7. Incidentally, the E_1/E_2 ratios for individual pregnancies (not shown) were in the relatively narrow range of 2.0–6.0. The average ratio of estriol to estrone plus estradiol (referred to hereafter as E_3/E_1+E_2) was in the range 7.0–9.0 at 20 and 25 weeks and then increased sharply to 13.0 at 30 weeks. This emphasizes the more rapidly increasing excretion of estriol at that particular time.

Detailed consideration of estrogen levels in the individual normal pregnancies is not merited here, but it is of interest to note the distinctly different E_3/E_1+E_2 ratios for different pregnant subjects. An example is shown in Table 1. One of these pregnant women (Subject A) had a low ratio of 3.0–5.0 during the greater part of the 20-week period, with a rise in the later stages, whereas the other (Subject B) had a much higher ratio throughout, with a similar tendency toward an eventual increase. In all of the 10 normal cases studied, there was a similar late and upward trend in this ratio, despite its variable magnitude.

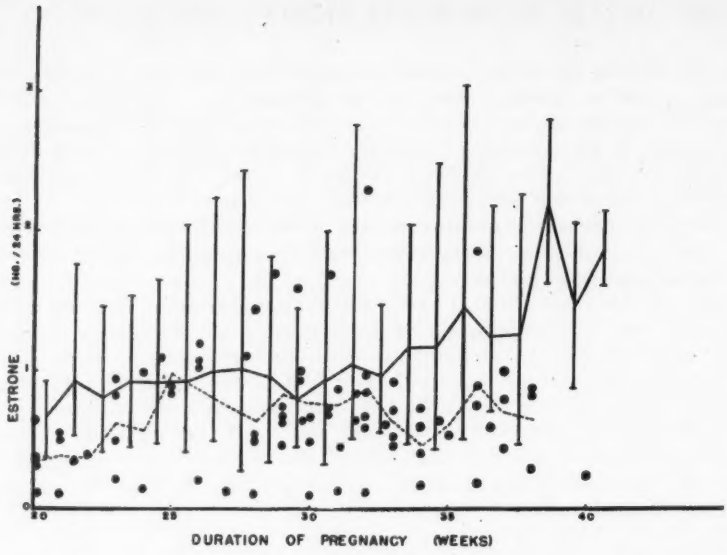

FIG. 1. Excretion of estrone during pregnancy in normal (———) and in diabetic (- - - -) subjects. Vertical lines represent the range of estrone measurements in 10 normal pregnant women, the mean weekly values being joined by an unbroken line. Each black dot represents an estrone measurement in any one of 14 diabetic subjects at various stages of pregnancy, the mean weekly values being joined by a broken line.

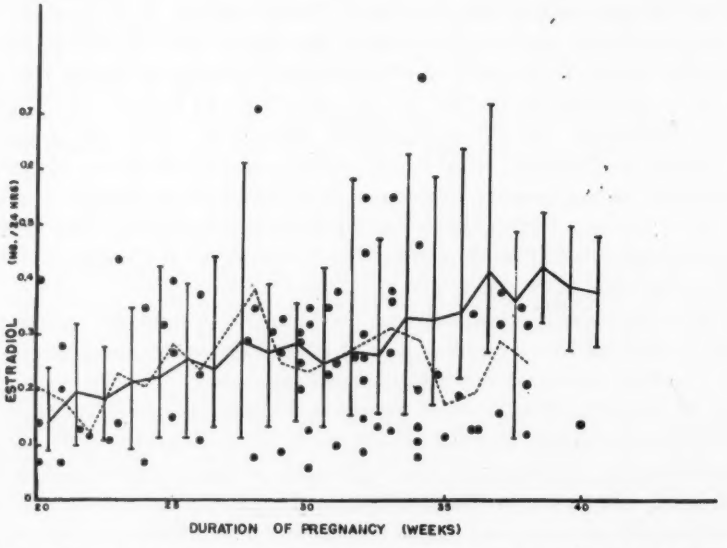

FIG. 2. Excretion of estradiol during pregnancy in normal (———) and in diabetic (- - - -) subjects. For key to other symbols (as applied to estradiol), see legend to Figure 1.

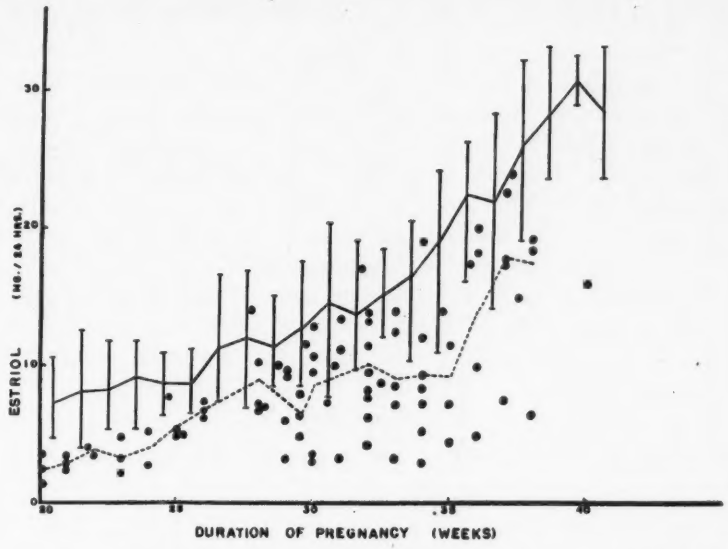

FIG. 3. Excretion of estriol during pregnancy in normal (———) and in diabetic (– – – –) subjects. For key to other symbols (as applied to estriol), see legend to Figure 1.

Diabetic pregnancy

In Figures 1, 2 and 3, the results for the 3 urinary estrogens in the diabetic group are shown along with results obtained in the normal group. This type of diagram was designed to show the range of urinary estrogen levels in the diabetic subjects, and not the trend of excretion in individuals.

Estrone and estradiol values in the diabetic group showed considerable scatter, but were mostly within the normal range, which was also wide. Individual determinations below the normal range in Figures 1 and 2 represent estrone and estradiol levels in 2 of the diabetic pregnancies studied. Estrone levels at 28 to 35 weeks in another diabetic patient (Subject C, Table 2; not included in Figure 1) were abnormally high. Omitting these high results, the average E_1/E_2 ratio for the diabetic group ranged from 3.2 to 3.5 when calculated at 20, 25, 30 and 35 weeks. This is similar to the ratio for the normal group. On the other hand, values for urinary estriol were almost wholly below the normal mean level, although about half of the results were within the normal range. The average E_3/E_1+E_2 ratio was lower in the diabetic group than in the normal women but the difference was not statistically significant. For the diabetic group, the ratio was in the range 5.3–7.6 between 25 and 30 weeks. As in the normal women, this ratio increased, and became 15.0 at 35 weeks. Thus there was a qualitative similarity between the two groups in this respect.

TABLE 1. URINARY E_3/E_1+E_2 RATIOS DURING 2 NORMAL PREGNANCIES

Subject	Week of pregnancy																	
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
A	4.1	4.4	4.4	3.8	3.1	3.8	3.9	3.3	4.9	—	4.9	5.0	6.4	5.3	7.9	5.0	10.0	11.6
B	19.1	16.0	21.3	20.0	14.8	16.1	14.5	15.7	16.5	18.6	20.0	19.4	19.0	12.9	21.1	23.8	26.6	—

TABLE 2. URINARY ESTROGEN EXCRETION AND RATIOS DURING 3 DIABETIC PREGNANCIES

Subject	Estrogen excretion (mg./24 hrs.) and ratios	Week of pregnancy														
		26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
C	Estriol			10.3	9.7	10.8	11.2	11.5	14.0	19.0						
	Estrone			5.2	4.5	5.1	4.6	6.0	4.8	6.0						
	Estradiol			0.35	0.33	0.32	0.38	0.55	0.55	0.77						
	E_3/E_1+E_2			1.9	1.2	2.0	2.2	1.8	2.6	2.8						
	E_1/E_2			14.9	13.6	16.0	12.1	10.9	8.7	7.8						
D	Estriol				3.2	3.1	3.2	4.2	3.2	2.9	4.5	4.9	7.7	6.5		
	Estrone				0.68	0.69	0.87	0.99	0.92	0.74	0.54	0.76	1.0	0.83		
	Estradiol				0.09	0.13	0.10	0.15	0.13	0.11	0.12	0.13	0.16	0.12		
	E_3/E_1+E_2				4.2	3.8	3.3	3.7	3.1	3.4	6.8	5.5	6.6	6.8		
	E_1/E_2				7.6	5.3	8.7	6.7	7.1	6.7	4.5	5.8	6.3	6.9		
E	Estriol	6.8		7.0		3.5		5.8		7.2		10.0		19.4		16.0
	Estrone	0.21		0.13		0.10		0.13		0.19		0.19		0.30		0.14
	Estradiol	0.11		0.08		0.06		0.09		0.08		0.13		0.21		0.14
	E_3/E_1+E_2	21.2		33.4		21.8		26.4		26.6		31.2		38.0		57.1
	E_1/E_2	1.9		1.6		1.7		1.4		2.4		1.5		1.4		1.0

Individual diabetic pregnancies

A few comments on the estrogen excretion pattern in individual diabetic patients are pertinent. In Subject C (Table 2) the urinary estrone level was extremely high despite the normal estriol and estradiol levels. This resulted in a greatly elevated E_1/E_2 ratio of 7.8–16.0 over the period 28–34 weeks. Although Subject D (Table 2) also exhibited an elevated E_1/E_2 ratio, this was due to a lowered excretion of estradiol rather than to a raised excretion of estrone; in addition, the excretion of estriol was low. Subject E showed a low excretion of estriol over the period 30–34 weeks, with a fairly late increase to near-normal levels. This was associated with low excretion of estrone and estradiol throughout the study period, to the extent that the E_3/E_1+E_2 ratio was always above 21.2 and rose to 57.0 at 40 weeks.

DISCUSSION

The average urinary E_1/E_2 ratio over the period 20–40 weeks of pregnancy is remarkably constant (3.2–4.7), and is also approximately similar in both diabetic and nondiabetic pregnancies. This suggests that these 2 estrogens arise from a common source in both pregnancy groups—probably placental estrone or estradiol or both—since a number of investigators have reported that administration of estrone or estradiol to nonpregnant human subjects results in similar urinary E_1/E_2 ratios (6, 8, 9). Furthermore, it is likely that at least part of the urinary estriol has the same origin, since the metabolic relationship between the 3 "classic" estrogens (estradiol⇌estrone→estriol) is well known. However, as has been pointed out by others (9, 10), during pregnancy a great deal more estriol is excreted relative to estrone and estradiol than can be accounted for by metabolic transformation of the latter 2 estrogens, unless these are metabolized to a different extent in pregnancy as compared with the nonpregnant state. In this connection it has been reported that the conversion of exogenous estradiol to urinary estriol is greater than normal in prostatic cancer (11), myocardial infarction (8) and mammary cancer (12). It has also been claimed that in pregnancy the high urinary estriol excretion can be attributed to a greater transformation of estradiol (13), but this finding could not be reproduced by Pearlman *et al.* (14) after administration of deuterium-labeled estrone acetate in pregnancy. If there is no difference in this respect between the pregnant and nonpregnant states it would seem that there must be another source of urinary estriol, independent of estrone and estradiol metabolism, in both normal and diabetic pregnancy. Some support for this concept has arisen from *in vitro* observations on placental and other tissues showing that estriol may be formed from androgens (15, 16) and perhaps by a route which does not involve estrone or

estradiol (17). Such a mechanism might explain the finding in Subject E (Table 2) of a markedly increasing estriol excretion with continuing low estrone and estradiol excretions.

A variable E_3/E_1+E_2 ratio is not by any means confined to diabetic pregnancies. Throughout 2 pregnancies uncomplicated by diabetes (Subjects A and B, Table 1), the values were strikingly different. This suggests a tendency for the pattern of estrogen metabolism to vary from one pregnant woman to another. Also, high E_1/E_2 ratios, whether due to a high excretion of estrone (Subject C, Table 2) or to a low excretion of estradiol (Subject D, Table 2), suggest the possibility of a change in the extent of interconversion between estrone and estradiol under certain circumstances. These observations indicate the need for renewed and extensive studies of estrogen biosynthetic pathways in pregnancy, and application of the findings to disorders of the pregnant state.

Acknowledgments

The authors wish to acknowledge the continued interest of Drs. E. H. Bensley, A. F. Fowler and J. S. L. Browne in this work. In the earlier stages of the study, assistance was rendered by Dr. R. M. Greenway, Miss Barbara Boon and Mr. John Damnovitz. Further technical assistance was provided by Miss M. Nilsen and Miss A. Metcalfe-Gibson.

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β-GLUCURONIDASE HYDROLYSIS OF URINARY CORTICOSTEROID CONJUGATES: THE EFFECT OF SALICYLATE GLUCURONOSIDE AS A COMPETING SUBSTRATE AND THE EFFECT OF ENZYME INACTIVATION*

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ABSTRACT

One microcurie of 4-C^{14}-cortisol was administered intravenously to 3 normal adults receiving 6–8 Gm. of acetylsalicylic acid daily, and urine collections were made at various intervals following the infusion. In some of these urine samples maximal liberation of radioactive glucuronoside was obtained only when using very high concentrations of a beef liver preparation of β-glucuronidase (3000 units per milliliter of urine) and with prolonged periods (five days) of incubation at 38° C. With a smaller amount of enzyme (400 units per milliliter of urine) and a shorter period of incubation (forty-eight hours at 38° C.), the degree of hydrolysis of corticosteroid glucuronosides was inversely related to the amount of salicylate glucuronoside present in the urine samples. The data suggest that salicylate glucuronoside acted as a competing substrate for the enzyme. When β-glucuronidase from beef liver was incubated at 38° C. with urine from normal subjects, there was an average loss of 62 per cent of the enzymatic activity after five days; approximately half of this loss occurred during the first twenty-four hours. The rate of enzyme inactivation did not appear to be altered by the presence of salicylate or its metabolites.

β-Glucuronidase has become a major tool in the hydrolysis of many of the urinary and plasma steroid conjugates and is used extensively in the routine determination of urinary 17-hydroxycorticosteroids (1). It is necessary that the limitations of such enzymatic hydrolysis be fully appreciated when quantitative estimations of the steroids freed by this procedure are performed. Several organic acids are known to be powerful competitive inhibitors of β-glucuronidase (2, 3) and certain macromolecular polyanions are thought to exert a similar effect, indirectly, through the inhibition of

Received October 9, 1959.

* This work was supported in part by research grants from the National Institutes of Health, United States Public Health Service, and in part by the Maryland Heart Association.

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enzyme activators (4, 5). Mammalian plasma has been shown to contain an unidentified, heat-stable, nondialyzable inhibitor in varying concentrations (6), and both dialyzable and nondialyzable inhibitors have been found in normal human urine (7). Of perhaps still greater importance to the liberation of a particular aglycone from urine by β-glucuronidase is the possible inhibitory effect of competing substrates which might be present in any random urine sample, as has been suggested by Levvy and Marsh (8).

In studies concerning the effect of salicylate administration on the metabolism and excretion of 17-hydroxycorticosteroids in man (9), it was found that when subjects received moderately large doses of acetylsalicylic acid the values for urinary corticosteroids were unexplainably low when these determinations were performed by the usual techniques employing β-glucuronidase hydrolysis. However, it was subsequently shown that when such urines were incubated with very large amounts of the enzyme, the steroid values rose well within the normal range.

It is the purpose of this paper to report a study of the inhibitory effect of a specific competing substrate upon the liberation of urinary corticosteroids by beef liver β-glucuronidase, in addition to certain observations concerning the rate of inactivation of β-glucuronidase when this enzyme was incubated with urine from normal human subjects.

METHODS

Urinary radiocorticosteroid conjugate fractions were determined by the methods described by Migeon *et al.* (10), after the intravenous administration of 4-C^{14}-cortisol to human subjects.

β-Glucuronidase activity was assayed, after addition of the enzyme to urine according to the method of Talalay *et al.* (11), in which phenolphthalein glucuronoside (Sigma)[1] was used as substrate.

Determinations of the urinary salicylate fractions (glycine and glucuronoside conjugates, as well as unconjugated salicylate) from subjects receiving acetylsalicylic acid were performed by the methods of Smith *et al.* (12).

The beef liver β-glucuronidase employed in this study was Ketodase (Warner-Chilcott), in an original concentration of 5000 Fishman units of β-glucuronidase activity per milliliter.

RESULTS

Effect of Varying Concentrations of Salicylate Glucuronoside upon β-Glucuronidase Hydrolysis of Urinary Radiocorticosteroid Glucuronosides

One microcurie of 4-C^{14}-cortisol was administered intravenously to each of 3 normal adult human subjects who were receiving moderately large

[1] The authors wish to express their appreciation to Mr. Dan Broida of the Sigma Chemical Company, St. Louis, Missouri, for his generous gifts of phenolphthalein glucuronoside.

amounts of acetylsalicylic acid (6–8 Gm. daily in fractional doses, for five days before, through two days after, the steroid infusion). The radioactivity released from urine incubated with varying amounts of β-glucuronidase for varying periods of time was determined. In Figure 1 it will be seen that as much as 3000 units of the enzyme per milliliter of urine was required to effect maximal hydrolysis of the glucuronoside fraction within a 48-hour incubation period at 38° C., and similar recoveries of radioactivity could be obtained after five days of incubation at the same temperature with one-third the amount of β-glucuronidase. The volume of each reaction mixture

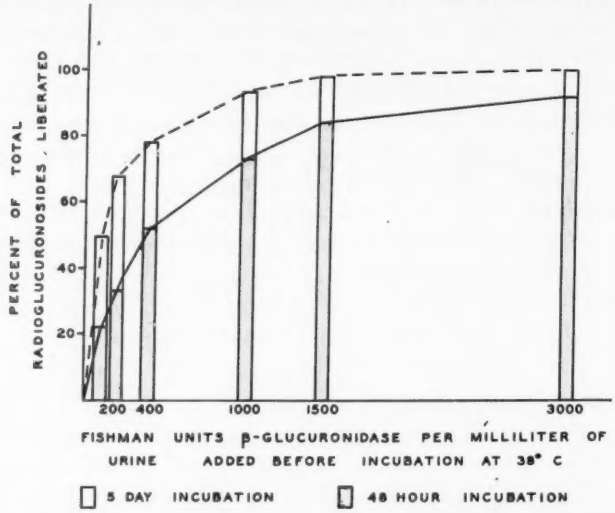

FIG. 1. Effect of concentration of β-glucuronidase and incubation-time (38° C., pH 4.6) on the liberation of radioactive corticosteroids in a urine sample (zero to four hours after the intravenous administration of 4-C^{14}-cortisol) from a subject receiving acetylsalicylic acid. This urine contained 0.64 mg. of salicylate glucuronoside per ml.

was adjusted with 2*M* acetate buffer so as to equal that containing the largest amount of the enzyme.

Curves of the type shown in Figure 1 were determined for 5 other urine samples from the same 3 subjects within the 48-hour period after the intravenous infusion of radiocortisol, and for a urine sample from 1 subject not being given salicylates to whom an identical dose of the radiosteroid had been administered. In all cases, maximal amounts of radioactivity were liberated after incubation of the urine for five days at 38° C. with 3000 units of β-glucuronidase per milliliter of urine. Comparable amounts of radioactivity were released after two incubations of forty-eight hours, each

preceded by the addition of more than 1200 units of β-glucuronidase per milliliter of urine. Values obtained using either of these procedures were considered to represent the *total* radiocorticosteroid glucuronoside content of a given urine sample.

It was noted that the amount of radioactivity released with smaller quantities of enzyme represented a greater percentage of the "total" steroid glucuronoside when the urine samples contained lower concentrations of salicylate glucuronoside. In Figure 2 is depicted the percentage of total glucuronoside radioactivity liberated after a 48-hour incubation with 400 units of β-glucuronidase per milliliter of urine compared with the salicylate glucuronoside concentration in 15 urine samples from the 3 subjects receiving salicylates. The hydrolysis of corticosteroid glucuronosides by this amount of β-glucuronidase was inversely related to the concentration of salicylate glucuronoside. This relationship suggests that the salicylate conjugate acted as a competing substrate, or that salicylate or its metabolites present in the urine had a more direct inhibitory action.

In an effort to separate both the free and conjugated corticosteroids from the salicylate glucuronoside, whole urine was subjected to extraction three times with one volume of ether:ethanol (3:1) after ammonium sulfate saturation, according to the technique described by Edwards *et al.* (13). The dry, pooled extracts were then taken up in distilled water and incubated with β-glucuronidase. The effect of variations in incubation-time, temperature, enzyme concentration and pre-extraction with ether:ethanol on the liberation of radioactive glucuronosides from a single urine sample containing 0.56 milligram of salicylate glucuronoside per milliliter is shown in Figure 3. It is apparent that maximal recoveries of radioactivity were obtained only with enzyme concentrations greater than 1500 units per milliliter of urine, unless longer periods of incubation were employed or unless the corticosteroid conjugates were extracted prior to hydrolysis. It must be pointed out that although this particular urine sample showed marked inhibitory effects upon the release of radioactivity by β-glucuronidase, determinations of residual enzyme activity in this urine after an incubation period of five days at 38° C., pH 4.6, showed that approximately 70 per cent of the initial amount remained (see also Fig. 6B).

Information was obtained regarding the rates of hydrolysis of the radiosteroid glucuronoside in relation to salicylate glucuronoside concentration, by the addition of 400 units of β-glucuronidase per milliliter of urine and performing chloroform extractions (1 volume × 3, each) at intervals throughout a five-day period of incubation at 38° C. In Figure 4, two such curves are shown which were determined on urine containing relatively large amounts of salicylate glucuronoside; these curves are compared with

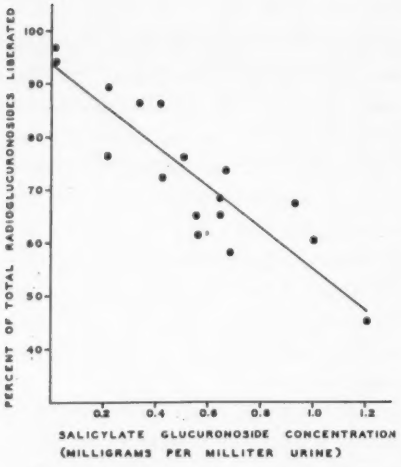

FIG. 2. Relationship between salicylate glucuronoside concentration and the liberation of radiocorticosteroids after incubation of urine with 400 units of β-glucuronidase for forty-eight hours at 38° C., pH 4.6.

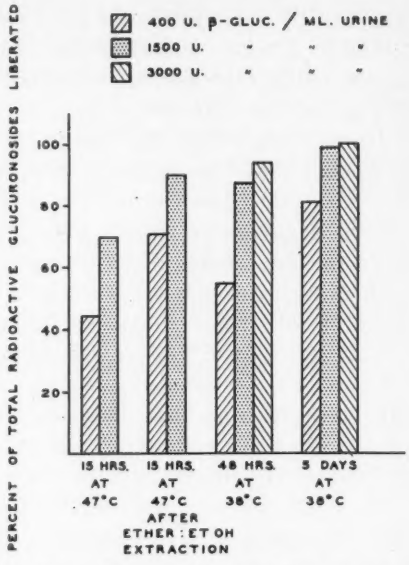

FIG. 3. Effect of incubation-time, temperature and concentration of β-glucuronidase on the liberation of radiocorticosteroids in a urine sample containing salicylate glucuronoside in a concentration of 0.56 mg. per ml.

the curve obtained from a urine sample known to be salicylate-free. It may be seen that during the first six hours of incubation, radioactive steroid glucuronosides were hydrolyzed in the salicylate-free urine at a rate four to six times greater than that determined in the 2 samples containing conjugated salicylates. Repeated chloroform extraction throughout the period of incubation did not interfere with β-glucuronidase hydrolysis, the cumulative radioactivity removed by this means being almost identical to that obtained from a single extraction (1 volume×3) at the termination of incubation.

Since free salicylate is removed by chloroform extraction, the data indicate that the inhibitory-like effect of these urines on the β-glucuronidase hydrolysis of corticosteroid glucuronosides could not be dependent upon inhibition of enzyme by the unconjugated salicylate liberated. Furthermore, and for the same reason, substrate equilibrium could not play a role in reducing the rate of hydrolysis. It would appear likely that the inhibitory effect observed was due to the presence of salicylate glucuronoside and that this conjugate acted as a competing substrate for the enzyme.

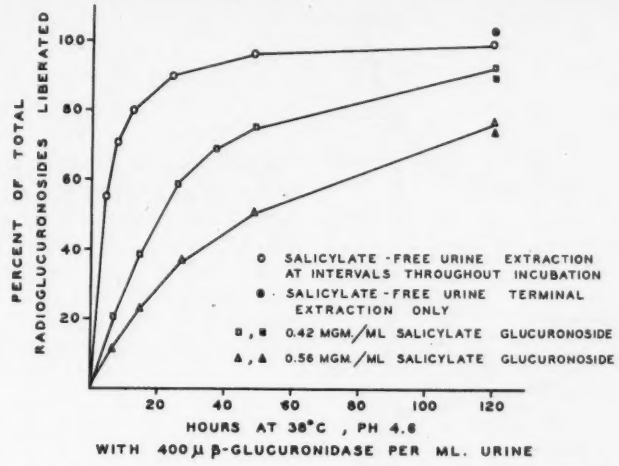

FIG. 4. Effect of salicylate glucuronoside concentration and interval chloroform extraction on the rate of β-glucuronidase hydrolysis of urinary radiocorticosteroid glucuronosides.

β-Glucuronidase Inactivation by Urine from Normal Subjects, and the Effect of Salicylate and Its Conjugates on the Hydrolytic Activity of Beef Liver β-Glucuronidase

Salicylate-free urine from normal subjects

When known amounts of beef liver β-glucuronidase were added to urine there was an almost immediate loss of enzyme activity as measured by the hydrolysis of phenolphthalein glucuronoside. Twelve random urine samples from normal human subjects varied considerably in this regard, the losses ranging from 1 to 30 per cent after an incubation period of five minutes at 38° C. (Fig. 5A). Although this initial apparent loss of enzyme activity may have been due to the presence of substances in urine which inhibited the hydrolysis of phenolphthalein glucuronoside in the assay itself, a progressive additional loss of enzyme activity occurred as the period of incubation was prolonged (Fig. 5A). There was considerable variation also in the rate of inactivation or decay of the enzyme from urine to urine; the average decrease in activity was 64 per cent after 120 hours at 38° C., pH 4.6, and about half of this deficit occurred within the first twenty-four hours. Multiple aliquots of a given urine yielded results which checked closely, as may be seen in Figure 5B. In all cases the pH remained constant throughout incubation, and there was no relationship between the decay in activity and urine osmolarity, as reflected by urine specific gravity. Thioglycolate cultures of random reaction mixtures, after incubation, failed to

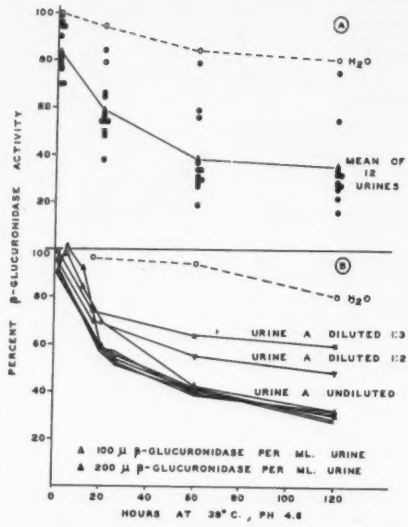

FIG. 5. Inactivation of β-glucuronidase by human urine. **A**) Decay of β-glucuronidase activity with time in 12 random normal human urine samples to which the enzyme had been added, compared to that observed when the enzyme was added to distilled water. **B**) Effect of dilution of urine on β-glucuronidase inactivation.

show bacterial growth. The effect of pre-boiling urine before the addition of enzyme was studied in only 1 instance; it was found that although there may have been a slight difference in the initial loss of activity, subsequent inactivation of the enzyme was similar to that seen in the unboiled duplicate samples.

The rate of decay in β-glucuronidase activity was not related to the amount of enzyme initially added to any particular urine sample. On the other hand, regardless of the absolute concentration per unit volume of urine, there was a proportionate decrease in the rate of β-glucuronidase inactivation as the urine was diluted with distilled water (Fig. 5B).

Effect of sodium salicylate added to urine from normal subjects

Sodium salicylate was added to urine in amounts calculated to produce concentrations of 1, 10, 50 and 100 micrograms per milliliter of urine. At none of these concentrations did the salicylate salt influence the rate of decay in enzyme activity within a given urine sample when this was assayed with phenolphthalein glucuronoside (Fig. 6A).

Effect of salicylate glucuronoside and salicyluric acid (glycine conjugate of salicylate)

Urines known to contain relatively large quantities of conjugated and unconjugated salicylate (collected from subjects receiving salicylates) showed no consistently greater inactivation of enzyme than did urines

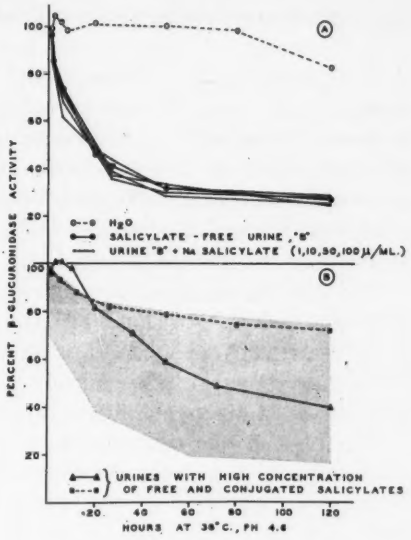

FIG. 6. Inactivation of β-glucuronidase added to urine containing salicylate and its conjugates. **A**) Values obtained for specimens of undiluted urine (normal subject) to which sodium salicylate had been added. **B**) Range of values (shaded area) for urine from normal adults compared to that for urine from subjects receiving moderately large doses of acetylsalicylic acid.

known to be salicylate-free. There seemed to be no relationship between the concentration of conjugated salicylate in urine and the percentage loss of added β-glucuronidase activity with time (five days at 38° C., pH 4.6), as is shown in Figure 6B. Similarly, urine samples containing large amounts of free and conjugated 17-hydroxycorticosteroids showed no greater β-glucuronidase inactivating qualities than did control urine from normal subjects.

DISCUSSION

The β-glucuronidase hydrolysis of urinary corticosteroid glucuronosides was strikingly impaired in the urine of subjects receiving moderately large doses of acetylsalicylic acid (aspirin). The data indicate that this inhibitory effect was directly proportional to the concentration of salicylate glucuronoside in such urine. Although it is possible that other conjugates of salicylate or metabolic products resulting from salicylate administration might function as β-glucuronidase inhibitors, it is postulated that salicylate glucuronoside acts as a competing substrate for the enzyme. It follows that a large variety of steroidal and nonsteroidal substances which are conjugated in this manner could have an inhibitory effect on the liberation of urinary corticosteroids by β-glucuronidase, if present in the urine in sufficient quantities. Pregnanediol glucuronoside, for example, might act as a potent competing substrate limiting the enzymatic hydrolysis of urinary corticosteroid conjugates, since there is reason to believe that the former

substance has a much greater affinity for the enzyme than does the latter (14).

In an investigation of the possible interrelationships between salicylate administration and the rate of utilization of corticosteroids, it was found that salicylates, when administered in moderately large doses, did not significantly alter the rates of disappearance from plasma or excretion in the urine of infused radiocortisol and cortisol (9). These findings are in agreement with those of Peterson *et al.* (15), who performed similar studies using an isotopic dilution technique. Done and Ely and their coworkers (16–18), on the other hand, reported a significant reduction in the urinary output of corticosteroids as determined by the method of Glenn and Nelson, in subjects receiving salicylates in a wide range of dosage. It is probable that the low values reported by this group were due to the inhibitory effect of the competing salicylate conjugate present in the urines so studied.

Inactivation of β-glucuronidase during incubation with urine did not seem to be related to the initial enzyme concentration, pH, specific gravity of the urine, bacterial growth, the presence of conjugated or unconjugated salicylates or corticosteroids, or the presence of chloroform, whereas the rate of inactivation appeared to be inversely proportional to the degree of pre-dilution of the urines studied. The pre-boiling of such urine produced only a negligible change in this degradation of the enzyme. It is probable that dilution decreased the concentration of inhibitors, but is also possible that dilution has a more direct inhibitory effect upon β-glucuronidase inactivators present in human urine, or that it enhances the potency of activating substances. In any event, the phenomenon would seem to outweigh the loss of enzyme activity that has been shown to result from dilution of the enzyme *per se* (19).

The observed inactivation of β-glucuronidase had little effect upon the net liberation of urinary corticosteroids reported here, for there was no apparent relationship between the percentage released and the residual β-glucuronidase activity. Furthermore, the concentration of salicylate glucuronoside was not related to the rate of inactivation of enzyme. This does not imply that inactivation of enzyme would not be a serious limiting factor in the hydrolytic yield of certain urine specimens, but it is probable that the inhibitory effect of a competing substrate is a much more important consideration.

It is difficult to determine the most efficient and economical method of β-glucuronidase hydrolysis for use in the routine estimation of urinary corticosteroids. Incubation for fifteen hours at 47° C. with 750 units of beef liver β-glucuronidase added per milliliter of urine is adequate in most instances, unless the concentration of competing substrate is extremely high.

Urine from patients known to be receiving aspirin in large doses might be subjected to prolonged incubation at higher enzyme concentrations or to ether:ethanol pre-extraction by the method of Edwards *et al.* (13)—a procedure suggested by Golub *et al.* (20) as a means of avoiding enzyme inhibitors in random urine samples. In prolonged incubation (five days at 38° C.) as employed by Gallagher *et al.* (21), the inactivation of β-glucuronidase might prove to be a serious limitation, and it is also possible that a portion of the Porter-Silber chromogen is degraded to non-phenylhydrazine-reactive material.

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GENERALIZED AND LOCALIZED (PRETIBIAL) MYXEDEMA: EFFECT OF THYROID ANALOGUES AND ADRENAL GLUCOCORTICOIDS*

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ABSTRACT

Administration of cortisone or related glucocorticoids resulted in a decrease in the quantity of metachromatic material in the lesions of pretibial and generalized myxedema. In generalized myxedema the rate of disappearance of the mucoprotein was greatest following the use of triiodothyroacetic acid and less rapid in the following order: triiodothyropropionic acid, *l*-triiodothyronine, and desiccated thyroid. In pretibial myxedema, the local injection or systemic administration of *l*-triiodothyronine appeared to be less effective than the adrenal glucocorticoids in altering the lesion.

THIS report is concerned with the effects of several thyroid analogues and of adrenal glucocorticoids on the lesions observed in generalized and localized (pretibial) myxedema. A previous study from this laboratory described the histogenesis of generalized myxedema and the sequelae of administration and withdrawal of desiccated thyroid in the human myxedematous subject (1–3). The current investigation extends these studies and in addition includes the lesions seen in localized myxedema. The latter lesions are of particular importance since they occur frequently in association with malignant exophthalmos and are deemed to have a similar pathogenesis.

METHODS AND MATERIALS

Patients with generalized or localized myxedema were studied. By means of an electrically driven hand drill equipped with bits, serial skin biopsies were carried out prior to and at frequent intervals during the course of administration of the test compound (1). In patients with generalized myxedema the lateral thigh was the site chosen for biopsy. In patients with pretibial myxedema, the biopsy specimen was taken from the site of the lesion. All specimens were taken in close proximity one to the other. The material obtained was fixed in alcohol-formalin and then stained with hematoxylin and

Received November 13, 1959.

* This work was supported by a research grant from the National Institutes of Health and by a grant-in-aid from the Warner-Chilcott Laboratories.

eosin, toluidine blue, and the Rinehart modification of the colloidal iron stain. The characteristic material found in both types of lesion is stained metachromatically with toluidine blue, and blue with the Rinehart stain.

The thyroidal analogues employed were triiodothyronine, triiodothyroacetic acid and triiodothyropropionic acid. The adrenal glucocorticoids were cortisone, cortisol and prednisone. When administered orally, the daily doses were as follows: *l*-triiodothyronine, 25–150 μg.; triiodothyroacetic acid, 6 mg. (fractionally); triiodothyropropionic acid, 5 mg.; prednisone, 20–30 mg.; and cortisone, 100 mg. By injection, cortisol or cortisone was given in a dose of 25 mg. Biopsy was performed after the local administration of 75–150 units of hyaluronidase.

The subjects included 3 patients with localized myxedema and 9 with generalized myxedema. Of the latter, 2 were treated with triiodothyroacetic acid, 1 with triiodothyropropionic acid and the remainder with triiodothyronine.

RESULTS

The characteristic material observed in pretibial myxedema was present in higher concentration and at a much deeper level in the skin than was the metachromatic material observed in generalized myxedema. Biopsy of the lesion in pretibial myxedema ten minutes after hyaluronidase had been injected into it revealed almost complete disappearance of the characteristic mucoprotein (Fig. 1, a and b), associated grossly and clinically with demonstrable pitting (Fig. 2). When a single dose of 25 mg. of cortisone or cortisol was injected into an untreated area of pretibial myxedema in each of the 3 subjects, the mucoprotein material decreased in quantity within a day, and progressive diminution was observable for at least fourteen days (Fig. 1, d and e). By inunction, cortisol (0.5 per cent) was weakly effective in the one subject in whom this route was employed. Prednisone when given in daily oral dose of 20 to 30 mg. to 2 of the patients induced a striking reduction in the amount of mucinous material in the pretibial lesions (Fig. 1, f and g). There was suggestive evidence that there might be some reaccumulation during the course of prolonged therapy (four weeks). When the administration of prednisone was discontinued, the lesion reverted to

FIG. 1. Histologic sections stained with toluidine blue:

a) Pretibial myxedema. Control for **b** and **c**. (×12.)

b) Pretibial myxedema; biopsy specimen obtained ten minutes after local injection of hyaluronidase. Note disappearance of metachromatic material. (×12.)

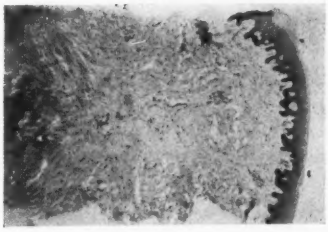
c) Pretibial myxedema. A dose of 40 μg. of triiodothyronine had been injected locally four days previously (×12.)

d) Pretibial myxedema. Control for **e.** (×12.)

e) Pretibial myxedema two days after local injection of 25 mg. of cortisol. Note decrease in metachromatic material. (×12.)

f) Pretibial myxedema. Control for **g.** (×30.)

g) Pretibial myxedema. A daily dose of 30 mg. of prednisone had been given by mouth for seven days (×30.)



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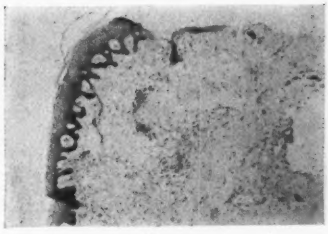

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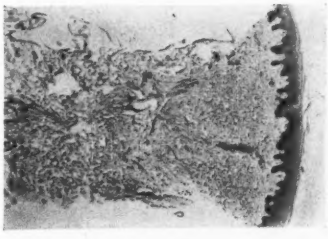

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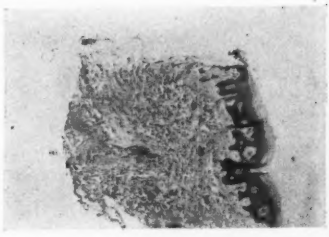

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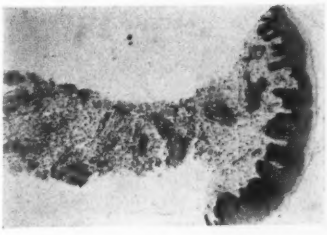

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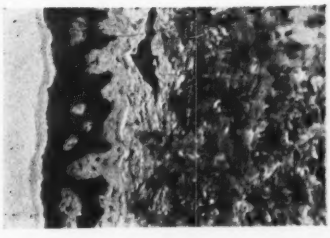

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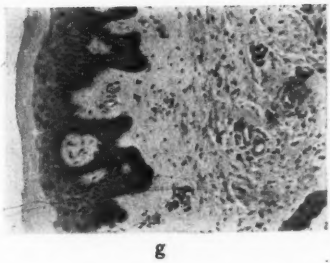

g

FIG. 2. Pretibial myxedema after local injection of hyaluronidase (right leg). Note area of pitting.

its former appearance. It is of interest that the material disappeared earliest in the deepest portion of the lesion.

In 2 patients, a single local injection of 40 or 100 μg. of *l*-triiodothyronine resulted in a decrease in the quantity of metachromatic-staining material for at least six days, although the decrease was far less than that seen following the use of cortisone (Fig. 1c). In 1 patient, the daily oral administration of 100 μg. of *l*-triiodothyronine also induced a decrease in the content of mucoprotein in the lesion. However, the daily oral administration of triiodothyroacetic acid for a period of three weeks was without effect in the 1 patient treated.

It is of interest that in patients with malignant exophthalmos the injection of hyaluronidase into a swollen lid will result in pitting (Fig. 3). The intra-orbital injection of hyaluronidase is, however, without definite permanent effect. Many of the symptoms of malignant exophthalmos, particularly the chemosis and lacrimation, are relieved by the oral administration of glucocorticoids. Their effects on exophthalmos have not yet been adequately defined and no experience has been accumulated in this laboratory as to the sequelae of intra-orbital injection of these agents.

In patients with generalized myxedema, the skin may be made to pit following the injection of hyaluronidase (Fig. 4). Indeed this phenomenon may be of aid in establishing the clinical diagnosis. It had previously been reported from this laboratory that following the administration of desiccated thyroid the metachromatic material decreased in quantity within two to three weeks and then disappeared over a period of six to eight weeks. In contrast, when thyroid therapy was stopped in the adequately treated patient with hypothyroidism, the material reaccumulated at a slower rate;

FIG. 3. Malignant exophthalmos. Hyaluronidase was injected into the left lower eyelid. Note the pitting.

indeed the change could not be definitely noted till three to five weeks had elapsed.

In the present studies it was observed that with triiodothyronine both the disappearance rate and the accumulation rate were somewhat more rapid. The time required for the former was less than a week and for the latter, two to four weeks. It is noteworthy that the mucoprotein disappears in a matter of days from the site of a local injection of triiodothyronine.

FIG. 4. Forearms of 3 patients with generalized myxedema and 1 normal subject (marked "normal"). Hyaluronidase was injected subcutaneously. Note the pitting at the site of injection in the 3 patients with myxedema, in contrast to the site of injection in the normal subject.

The action of triiodothyroacetic acid (2 subjects) was even more prompt than that of triiodothyronine. When triiodothyroacetic acid was given orally, the mucoprotein was reduced strikingly or even disappeared within seven days, and when the drug was stopped reaccumulation occurred within the same period.

The action of triiodothyropropionic acid (1 subject) was intermediate between that of triiodothyroacetic acid and triiodothyronine. A marked decrease in the metachromatic material was seen after seven days of therapy, and when the drug was withdrawn striking reaccumulation was observed within fourteen days.

As in patients with pretibial myxedema, the oral administration of cortisone in a dosage of 100 mg. per day to patients with primary myxedema resulted in a prompt decrease in the amount of metachromasia within a matter of days.

DISCUSSION

The existence of an extracellular interfibrillar mucinous substance in the dermis and other connective tissues of patients with a deficiency of thyroid hormone has been well known for many years. However, it remained for Watson and Pearce (4) in their investigations of localized myxedema to demonstrate that the extracellular material present in these lesions was composed of the acid polysaccharides, chondroitin-sulfuric acid and hyaluronic acid, mixed with or combined in some form with proteins. It seems likely that the material in generalized myxedema is of a similar nature. However, the material seen in localized myxedema differs from that in generalized myxedema with respect to density, depth of staining, and location. In pretibial myxedema the lesion is deep in the dermis, whereas in generalized myxedema it is superficially located in the upper papillary layers. However, in both lesions the local injection of hyaluronidase results in gross pitting within a few minutes, and disappearance of the characteristic material histologically. *In vitro*, also, the mucopolysaccharide can be made to decrease or disappear, but only after incubation with hyaluronidase for a period of hours.

These studies demonstrate the effectiveness of triiodothyroacetic acid, triiothyropropionic acid and triiodothyronine in causing the disappearance of generalized myxedema. The sequence in which they are listed indicates the relative rapidity of action, with triiodothyroacetic acid acting the most rapidly. However, it should be noted that the data for triiodothyroacetic acid were obtained on only 2 patients and the data for triiodothyropropionic acid on only 1 patient. Either a single local injection or the oral administration of triiodothyronine reduced the amount of metachromatic-staining material in the lesion of localized myxedema. While these studies

were in progress (3), Warthin and Boshell (5) reported satisfactory treatment of such lesions with repeated injections of triiodothyronine in 1 patient. In contrast, triiodothyroacetic acid administered by mouth failed to alter the pretibial lesion. However, triiodothyronine was far less effective in the treatment of pretibial myxedema than were the adrenocortical glucocorticoids. These latter agents, whether given by local injection or by mouth, resulted in dramatic and prolonged improvement of the localized myxedematous lesions. Several other investigators have treated cases of localized myxedema with similar improvement (6–9). It is not surprising that these adrenal steroids also reduce the amount of mucoid material demonstrable in generalized myxedema.

The importance of these studies on pretibial myxedema lies in the possibility that the knowledge gained may be applied to the treatment of malignant exophthalmos. These two lesions are frequently associated and are presumed to have a common pathogenesis. Although the swollen eyelid of the patient with malignant exophthalmos can often be made to pit by the use of hyaluronidase, the phenomenon is of short duration. The intra-orbital injection of this enzyme has been found by us and by others (10) to be of little, if any, value perhaps because of the short duration of action. However, it must be realized that although the early lesion in malignant exophthalmos may consist of excessive amounts of mucoprotein and bound water, at a later date replacement fibrosis may be present. It is well known that after a period of years the progression of proptosis ceases spontaneously.

Cortisone has been reported to be of value in the treatment of malignant exophthalmos in some of the relatively few patients so treated (11, 12). The chemosis and lacrimation have usually been helped and at times the exophthalmos has been reduced. Here, too, the best results have been observed in the early cases. Only 2 cases treated with intra-orbital cortisone have been reported, and these were not attended with success (7). In view of our findings, it would seem worth while to study further the effectiveness of intra-orbital injection of cortisol or related compounds in the treatment of malignant exophthalmos. Perhaps the best results may be anticipated in patients in whom the injection of hyaluronidase into the eyelid results in pitting.

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THE ROLE OF HORMONES IN HUMAN BEHAVIOR. III. CHANGES IN FEMALE SEXUALITY AFTER HYPOPHYSECTOMY*

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ABSTRACT

A study was made of the sexual functioning of women with metastatic breast cancer who had undergone hypophysectomy as a therapeutic measure to check the progress of the disease. The study was divided into two phases. In the first phase, 20 women, mean age 51 years, were interviewed before and after hypophysectomy with regard to their sexual functioning. In the second phase, 30 women, mean age 51 years, were studied after hypophysectomy. Emphasis was placed on three aspects of sexual behavior, *i.e.*, desire, activity and gratification. Of the 30 women in the second phase, 23 had previously undergone mastectomy, and 7 oophorectomy; the sexual reactions to all three types of surgery were investigated. Results indicated that neither mastectomy nor oophorectomy had a significant influence on the sexual functioning of these women, whereas hypophysectomy resulted in a sharp decrease of sexual desire, activity and gratification. It was concluded that absence of the tropic pituitary hormone which activates the adrenal androgens (seemingly the only source of androgen in women) accounted for the decline in sexual functioning. This conclusion was based on the following: 1) the postoperative administration of thyroid hormones or of cortisone had no effect on the sexual status after surgery; 2) results of studies reported in the literature indicate that deprivation of ovarian hormones does not appreciably affect sexual behavior. Thus, lack of adrenal androgens appears to be the chief cause of the sharp drop in female sexual activity after hypophysectomy.

INTRODUCTION

THE effect of hormones on sexual behavior has been a controversial issue for some time. Many research studies support the theory that sexual behavior in humans is mainly dependent upon gonadal hormones (1–3), whereas others stress primarily the importance of psychologic factors only (4–6). Beach (7) advocates a compromise.

Received July 17, 1959.

* This study was supported in part by research grant M1118 from the National Institute of Mental Health, U. S. Public Health Service.

Gratitude is expressed to Dr. Olof H. Pearson and Dr. Ernest J. Greenberg for their assistance in obtaining the patients for this study and to Dr. Sheldon E. Waxenberg for his helpful suggestions in discussing this paper.

Conclusions on the relationship between reduced pituitary functioning and sexual reactions have been mainly based on animal experiments (8). Recently, however, a unique opportunity has presented itself to study directly the psychologic reactions in humans to the extirpation of the pituitary gland (9–12). The present study concerns itself only with the effects of hypophysectomy on the sexual functioning of women.

Hypophysectomy is now performed in an attempt to induce remission of certain carcinomas suspected to be dependent upon the action of pituitary hormones (13). Indications and contraindications for this type of surgery have been amply documented in the literature (14–16). Following hypophysectomy, those hormones that are absolutely necessary for the maintenance of the functions of the living body are replaced. Cortisone is given before, during, and after surgery. Thyroid hormones are administered as soon as hypothyroidism becomes clinically apparent, usually at four to eight weeks after hypophysectomy. Pitressin is administered soon after surgery in order to inhibit polyuria. After hypophysectomy, menstruation ceases and the vaginal epithelium and endometrium become atrophic. Urinary gonadotropin becomes unmeasurable.

PROCEDURE

Phase I

This investigation was carried out in two phases. During Phase I, 20 women with metastatic breast cancer who were considered for hypophysectomy were interviewed before surgery and six to ten weeks after hypophysectomy, regarding their psychologic reactions to this operation. Their ages ranged from 40 to 66 years (mean age, 51 years). In some of the patients there had already been interference with normal endocrine function prior to hypophysectomy. Six women had undergone oophorectomy, and 2 radiation castration. Of these 8 castrated patients, 3 had subsequently been adrenalectomized.

All patients, except one who improved physically, reported after hypophysectomy either a decrease or abolition of sexual desire. Patients who abstained from sexual relations because of the husband's advanced age or impaired health, or for other reasons, were excluded. This exclusion left 9 patients who could be evaluated. Of these, 1 denied any change in sexual functioning, and 8 stated that their sex desire had been abolished or decreased. They resumed sexual intercourse only in order to please their husbands. Three women had reported cessation of sexual desire after the antecedent adrenalectomy; it was then assumed that some adrenocortical hormones other than cortisone play a significant role in maintaining sexual behavior in women (9). Since these 8 patients at the time of post-hypophysectomy evaluation were in a state of secondary hypothyroidism, the sexual change might have been related to thyroid deficiency. This necessitated Phase II of the investigation.

Phase II

A study was made of a separate series of 30 women with metastatic breast cancer who had undergone hypophysectomy to arrest the disease. For reasons beyond our control, these patients could not be evaluated before surgery. Their ages ranged from 31 to 65

years (mean age, 51 years). Twenty-four patients were married, 2 widowed, 3 divorced or separated, and 1 was single. Twenty-three patients had previously undergone simple or radical mastectomy, and 7 had inoperable breast carcinoma. Of these 23 patients, 7 subsequently had been oophorectomized. None had been adrenalectomized.

All were treated with thyroid substitution hormones and cortisone. They were interviewed two to thirty months (mean interval, ten months) after hypophysectomy regarding their preoperative and postoperative sexual behavior involving all three types of surgery, namely, mastectomy, oophorectomy and hypophysectomy. Emphasis was placed on: 1) sexual desire, 2) heterosexual activity in terms of frequency of intercourse, and 3) sexual responsiveness, *i.e.*, the degree of gratification obtained from sexual contact. In 18 patients there was objective improvement after hypophysectomy, as shown by regression or arrest of growth of the tumor (13, 16); in 3 the response was subjective; and in the remaining 9 there was symptomatic improvement. All patients felt well enough to perform their household duties or carry on with their occupations.

RESULTS

Reactions to mastectomy

Sexual desire. Before mastectomy, 22 patients had desire and 1 had none. The latter patient was separated from her husband and denied any sexual desire, activity or gratification of any kind after separation. After surgery, 19 retained the same degree of desire, 2 reported a slight decrease and 1 was uncertain since she did not resume sexual activity because she felt too embarrassed with only one breast.

Sexual activity. Twenty-one patients led an active sexual life before surgery. Of these, 18 resumed sexual activity after the operation. Two patients discontinued sexual relations; one of them felt too disfigured, and the other patient's husband was afraid of injuring the operative site. From 1 unmarried patient no adequate information could be obtained (classification, "uncertain").

Sexual gratification. Of the 21 patients who had sexual intercourse before surgery, 20 had experienced orgasm, and 1 was too tired both before and after surgery to know if she experienced pleasure (classification, "uncertain"). After the operation, 15 reported gratification to the same degree, 2 to a lesser extent, and 1 had no sexual sensations. The 2 patients who did not resume sexual relations denied satisfaction of any kind.

In summary, 85 per cent of the women retained the same degree of sexual desire and frequency of activity after mastectomy as before, and 75 per cent experienced the same intensity of gratification. These findings are recorded in Table 1.

Reactions to oophorectomy

Seven patients had undergone oophorectomy but information was obtained from only 6.

Sexual desire. Before surgery, all 6 women had sexual desire, led an ac-

tive sexual life and experienced orgasm. After oophorectomy, desire remained at the same level in 4 patients, in 1 it decreased mildly, and in 1 it dropped to zero.

Sexual activity. Three patients retained the preoperative frequency of intercourse and 3 reported some decrease.

Sexual gratification. Four of the 6 women experienced the same degree of sexual pleasure after oophorectomy; in 1 the sensation was less intense, and in 1 there was no sensation.

TABLE 1. PREMASTECTOMY AND POSTMASTECTOMY SEXUAL FUNCTIONING OF 23 WOMEN

Degree of experience	Number of women experiencing					
	Sex desire		Sex activity		Sex gratification	
	Preop.	Postop.	Preop.	Postop.	Preop.	Postop.
Normal	22	19	21	18	20	15
Decreased		2		1		2
Uncertain		1	1*	1*	2 (1+1)*	2 (1+1)*
Zero level	1	1	1	3 (1+2)	1	4 (1+3)
Totals	23	23	23	23	23	23
Percentage of patients retaining preoperative level	86.4		85.7		75.0	
Percentage of patients experiencing postoperative						
Decrease	13.6	13.6	4.7	14.3	10.0	25.0
Abolition	0		9.6		15.0	
Totals	100%		100%		100%	

* This patient was single and could not be adequately evaluated in every respect (see text).

Although the number of cases is too small for statistical evaluation, it is probable that oophorectomy had little effect on sexual functioning in 4 of the 6 women. The findings are summarized in Table 2.

Reactions to hypophysectomy

Sexual desire. Twenty-five of the 30 women had sexual desire in various degrees before hypophysectomy, and 5 had none. After surgery, there was a marked decrease in desire in 1 patient, a mild decrease in 2, and a drop to zero level in 21. One patient who had no desire before operation because

she was too ill, experienced some libido after hypophysectomy when she felt physically well (classification, "uncertain"). The 5 patients who had no desire before hypophysectomy had no desire afterward. The following statements reflect vividly the patients' feelings after surgery: "No desire, 'No' with capital letters." "It dropped like a hot potato; not as much as *that*" (snapping her fingers to illustrate the degree of desire).

Sexual activity. Before hypophysectomy, 23 of the 30 women were sexually active in varying frequency, and 7 were inactive. After surgery, 12 patients remained active; of these, 3 were active at the same rate, and 9 at less frequent intervals; 10 continued to have intercourse despite absence of desire, mainly in order not to deprive their husbands of their marital rights.

TABLE 2. PRE-OOPHORECTOMY AND POST-OOPHORECTOMY SEXUAL FUNCTIONING OF 6 WOMEN

Degree of experience	Number of women experiencing					
	Sex desire		Sex activity		Sex gratification	
	Preop.	Postop.	Preop.	Postop.	Preop.	Postop.
Normal	6	4	6	3	6	4
Decreased		1		3		1
Uncertain						
Zero level		1				1
Totals	6	6	6	6	6	6
Percentage of patients retaining preoperative level	66.6		50.0		66.6	
Percentage of patients experiencing postoperative:						
Decrease	16.7	33.4	50.0	50.0	16.7	33.4
Abolition	16.7		0		16.7	
Totals	100%		100%		100%	

Sexual gratification. Of the 23 patients who led an active sexual life before hypophysectomy, 20 had orgasm. After surgery only 5 patients experienced pleasure, 3 of them to the same degree and 2 less intense. Three of these 5 patients had no sexual desire, but once they decided to have intercourse they also obtained gratification.

Though sexual functioning decreased after hypophysectomy, the pa-

TABLE 3. PREHYPOPHYSECTOMY AND POSTHYPOPHYSECTOMY SEXUAL FUNCTIONING OF 30 WOMEN

Degree of experience	Number of women experiencing					
	Sex Desire		Sex activity		Sex gratification	
	Pre-op.	Postop.	Pre-op.	Postop.	Pre-op.	Postop.
Normal	25		23	3	20	3
Decreased		3		9		2
Uncertain		1				
Zero level	5	26 (5+21)	7	18 (7+11)	10	25 (10+15)
Totals	30	30	30	30	30	30
Percentage of patients retaining preoperative level	0		13		15	
Percentage of patients experiencing postoperative:						
Decrease	16		39		10	
Abolition	84	100	48	87	75	85
Totals	100%		100%		100%	

tients themselves did not feel this deficiency keenly. They were only concerned about its effect on their husbands.

In summary, after hypophysectomy none of the patients retained the same degree of preoperative sexual desire. In 87 per cent, sexual activity was less frequent or absent. In 85 per cent, sexual gratification was reduced or not experienced. These data are summarized in Table 3.

DISCUSSION

This study was an attempt to evaluate the sexual functioning of women from the time they were faced with the diagnosis of breast cancer through the period covering the various operations related to this disease. Results indicate that mastectomy did not appreciably influence sexual behavior. Bard and Sutherland (17) in their study on the adaptation to radical mastectomy found that sexual difficulties can arise because of a feeling of mutilation or fear of injury to the operative site. Such feelings were also observed in our series.

Only 6 or our patients underwent oophorectomy, so the results of this operation are difficult to evaluate. Nevertheless, oophorectomy appeared

to have no appreciable effect on their sexual behavior. The findings are in agreement with the current consensus surveyed by Kinsey *et al.* (18), that neither the natural nor the surgical menopause necessarily influences the sexual behavior of women. Filler and Drezner (19) also reported that of 41 women under the age of 40 who had undergone oophorectomy, 36 experienced no change in sexual desire.

Findings in our hypophysectomy patients indicate that extirpation of the pituitary gland resulted in a fairly drastic decrease of sexual functioning whereby sexual desire and gratification were mainly affected. Removal of the pituitary deprived the patients of pituitary hormones which stimulate the thyroid, the gonads and the adrenals. It would be interesting to determine which of these target organs, and which of their respective hormones, account for the sharp decrease in adequate sexual functioning. Since the administration of exogenous thyroid hormones did not induce a resurgence of sexual functioning, it can be assumed that thyroid deficiency was not the cause of this change. Gonadal hormones were not administered; however, according to current opinion (18) the absence of ovarian hormones does not necessarily influence sexual behavior, so it is unlikely that deprivation of estrogens of ovarian origin accounted for the drastic decrease in sexual desire and responsiveness. The adrenal cortex produces cortisone and a variety of other hormones including androgens and estrogens. Cortisone was administered to these patients after hypophysectomy, but no return of sexual functioning could be observed. We can therefore exclude deprivation of cortisone as a cause for the decrease of sexual functioning. Waxenberg *et al.* (20) studied a series of women with metastatic breast cancer who underwent oophorectomy and adrenalectomy. They observed a significant decrease in sexual activity after adrenalectomy. Evidence was presented that the adrenal androgens rather than the estrogens accounted for this decrement.

Our findings were similar to those of Waxenberg *et al.* relative to the drastic reduction in sexual expression following hypophysectomy. In Waxenberg's patients, the ovaries and adrenals (which are the major source of estrogens and androgens) were removed, but the pituitary gland was left intact. In our Phase II series, only a few patients had been ovariectomized but none of them had been adrenalectomized; thus major sources of estrogen and androgens were still present. All of them, however, were deprived of the pituitary gland and, in turn, of the tropic hormones without which the ovaries and adrenals atrophy. It would seem, therefore, that cessation of the secretion of adrenal androgens due to removal of the pituitary gland accounted for the reduction in sexual functioning.

It has been known for some time that exogenous androgens stimulate libido and increase sexual activity in women (21–24). A study carried out

recently at our institution by Sopchak (25) supports these findings. Administration of androgens effected a significant increase in sexual desire but not in frequency of intercourse.

It is noteworthy that neither in Sopchak's group nor in ours was sexual desire related to frequency of intercourse. Apparently sexual intercourse is independent of hormonal factors and is more related to psychologic factors.

One could object to our interpretation and attribute the changes in sexual behavior to progression of the disease or to physical impairment. More than half of the patients, however, were classified as objectively improved and all experienced a subjective remission and felt comparatively well. Since the interviews were retrospective, one could also question the reliability of the patient's information. Nevertheless, their feelings were so vividly expressed that it is difficult to doubt or discount their reports. It can therefore be concluded that there is probably a relationship between the decrease in sexual functioning and the absence of adrenal androgens caused by the extirpation of the pituitary gland.

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ENDOCRINOLOGIC AND METABOLIC EFFECTS OF 17α-METHYL-19-NORTESTOSTERONE IN WOMEN*†

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ABSTRACT

Endocrinologic and metabolic effects of 17α-methyl-19-nortestosterone (17-MNT) and testosterone propionate (TP) were determined in 40 castrated or postmenopausal women with advanced metastatic carcinoma of the breast. In patients receiving 17-MNT there was retention of fluid, virilization and withdrawal uterine bleeding. A significant fall in the level of serum protein-bound iodine occurred early in therapy, associated with a decrease in thyroxine-binding protein and an increase in the basal metabolic rate. The level of serum cholesterol increased to abnormal values in patients treated eight weeks or longer. The urinary excretion of 17-ketosteroids and of 17-hydroxycorticosteroids fell significantly, but plasma 17-hydroxycorticosteroid levels were unchanged. Urinary gonadotropin excretion was suppressed. Serum bilirubin concentration rose to abnormal values in all patients receiving more than 4200 mg. of 17-MNT, but there was no change in the results of other routine liver function tests. Apart from a decrease in the level of serum citric acid, there was no alteration in the chemical constituents of either blood or urine. Patients receiving TP showed less fluid retention and greater virilization. The fall in the level of serum protein-bound iodine with TP occurred later and was of lesser magnitude than that observed with 17-MNT. The urinary excretion of 17-ketosteroids increased, but urinary and plasma 17-hydroxycorticosteroids were unchanged. Urinary gonadotropin excretion was suppressed. Hypokaliemia and hypercalcemia occurred in 1 subject each.

Received December 2, 1959.

* This work was supported in part by Grants CY3601 and CY3358 from The National Cancer Institute, The National Institutes of Health, United States Public Health Service.

† These studies were carried out in the course of a Cooperative Study to Evaluate Experimental Steroids in the Therapy of Advanced Breast Carcinoma sponsored by the Cancer Chemotherapy National Service Center, which supplied the 17α-methyl-19-nortestosterone (Parke, Davis & Company) and testosterone propionate. The cooperation and assistance of the staff of the Cancer Chemotherapy National Service Center is gratefully acknowledged.

STUDIES of the clinical, metabolic and endocrinologic effects of 17α-methyl-19-nortestosterone (17-MNT) and of testosterone propionate (TP) were carried out on 40 women with advanced progressive metastatic carcinoma of the breast treated with one or the other drug according to the randomized scheme outlined by Segaloff (1).

MATERIALS AND METHODS

The 40 patients were castrated or postmenopausal women, studied so far as possible without selection. Most were ambulatory out-patients. Details of the chronologic age, stage in the menopause, and dosage, mode of administration and duration of therapy are summarized in Table 1. The patients were examined at intervals of two to four weeks, and they were followed and studied until therapy was discontinued because of the progression of cancer or the appearance of adverse effects attributed to the hormone therapy. Three women died while receiving 17-MNT and 4 died while receiving TP.

The following determinations were made: serum and urinary calcium (2), phosphorus (3) and citric acid (4); serum alkaline phosphatase (5), cholesterol (6), bilirubin, total protein, albumin, globulin, sodium, potassium, protein-bound iodine (7, 8); blood urea

TABLE 1. SUBJECTS AND STEROIDS EMPLOYED

		17-MNT (10 mg. thrice daily, orally)	TP (100 mg. thrice weekly, i.m.)
No. of patients, according to age groups	30–39	1	1
	40–49	2	6
	50–59	6	3
	60–69	6	5
	70+	5	5
Range of age (yrs.)		39–79	35–82
Mean age (yrs.)		61	59
No. of patients, according to stage in menopause (yrs.)	>10	12	11
	6–10	3	2
	1– 5	2	3
No. of patients castrated (less than 1 yr.)		3	4
No. of patients according to duration of treatment (wks.)	2– 6	5	5
	7–12	9	4
	13–19	1	3
	20+	5	8
Mean duration of treatment (wks.)		13	22
Range of cumulative dose (mg.)		450–6610	1000–19600

nitrogen and sugar; plasma 17-hydroxycorticosteroids (9); urinary gonadotropins (10), 17-hydroxycorticosteroids (9), 17-ketosteroids (11), and creatinine (12); 24-hour thyroidal I^{131} uptake; and basal metabolic rate.

RESULTS

General clinical effects

The effect of these steroids on mammary carcinoma in women is reported elsewhere (13).

In 11 of 20 patients receiving 17-MNT, there was retention of fluid, manifested by rapid weight gain and edema; a weight loss of from 5 to 22 pounds occurred following administration of Mercuhydrin and/or chlorthiazide (Diuril).[1] In 7 patients hirsutism, deepening of the voice, acne, and/or clitoral hypertrophy were noted. Alopecia occurred in 3. Vaginal bleeding was observed by 5 of 13 women with intact uteri who survived more than five days after the termination of treatment.

In 8 of the 20 subjects receiving TP, there was retention of fluid. In 10 women hirsutism, deepening of the voice, acne, clitoral hypertrophy, increased libido, dyspareunia and/or excessive vaginal discharge were observed.

Thyroid function

In 16 of the 20 women receiving 17-MNT, various parameters of thyroid function were studied serially. Results in a representative case are shown in Figure 1. In all patients there was a fall in the level of serum protein-bound iodine (PBI), manifest after two weeks of treatment; the level remained depressed throughout the course of treatment. The control PBI values and the lowest values obtained during therapy in the 16 patients are presented in Table 2; the dosage of 17-MNT is the cumulative amount which the patient had received by the time the lowest value for PBI was observed. The differences were statistically significant ($P<.001$). With discontinuation of 17-MNT, the concentration of PBI immediately began to rise slowly to the control level, which was reached in ten weeks.

Thyroid function was studied in 9 of the 20 women given TP. There was a fall in level of serum PBI during therapy, which was manifest at two weeks and maximal after seventy-one days of treatment. The control and the lowest PBI values for these 9 patients are presented in Table 2. The differences were statistically significant ($P<.01$).

In the 3 patients of the 17-MNT group in whom serum thyroxine-binding protein (TBP)[2] was studied, the level fell during therapy with 17-MNT.

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[1] We are indebted to Dr. Elmer Alpert of Merck Sharpe and Dohme for supplying us with chlorthiazide (Diuril).

[2] Studies performed through the courtesy of Dr. Jacob Robbins, National Institute

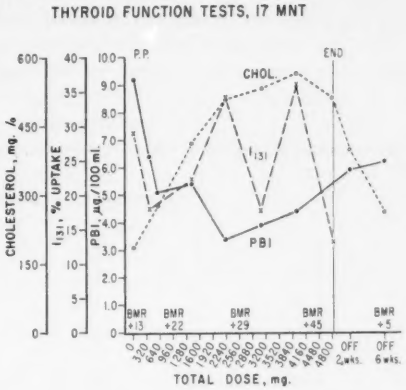

FIG. 1. Levels of serum protein-bound iodine (PBI) and cholesterol, 24-hour thyroidal uptake of I^{131} and basal metabolic rate (BMR) determined serially in a 54-year-old postmenopausal Negress with progressive metastatic carcinoma of the breast involving the bones. This patient received 17α-methyl-19-nortestosterone, 30 mg. daily for five months (total dose, 4800 mg.). Objective regression of bone lesions occurred. Therapy was stopped because of jaundice.

Individual determinations of TBP and the corresponding values for PBI are presented in Table 3. The concentration of free thyroxine during therapy was increased three-fold over control values (14).

The 24-hour thyroidal I^{131} uptake averaged 25 per cent in 11 untreated patients; during subsequent treatment with 17-MNT, uptake values averaged 22 per cent. In another group of 7 untreated patients, uptake values averaged 38 per cent and were unchanged during therapy with TP (mean value, 35 per cent).

The levels of serum cholesterol in 13 patients throughout the course of therapy with 17-MNT are depicted in Figure 2. In 8 patients treated for six weeks to seven months, mean serum cholesterol concentration increased from a control level of 238 mg. per 100 ml. to a peak value of 380 mg. per 100 ml. Hypercholesteremia was manifest at eight weeks and maximal at fifteen weeks. After discontinuation of 17-MNT, the elevated levels of cholesterol returned to pretreatment values in two to six weeks. In the remaining 5 patients treated with 17-MNT for a shorter period (six to eleven weeks) mean levels of serum cholesterol were unchanged. In 7 other patients treated with TP, the mean concentration of serum cholesterol was 244 mg. per 100 ml. prior to therapy and 264 mg. per 100 ml. during therapy. The concentration rose in 2 patients, was unchanged in 4 and decreased in 1.

The basal metabolic rate was determined serially in 2 patients given 17-MNT. Control values of +13 and +4 per cent rose to peaks of +40 and +30 per cent during two and six months of therapy, respectively. Six weeks after discontinuation of 17-MNT, the BMR in 1 of these subjects was +5 per cent (Fig. 1).

of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service.

TABLE 2. LEVELS OF SERUM PROTEIN-BOUND IODINE

Patient	PBI (μg./100 ml.)		Total dose of steroid (mg.)
	Control level	Lowest level	
17α-Methyl-19-nortestosterone, 30 mg. daily, by mouth			
T.W.	10.4	3.8	3060
F.R.	6.1	3.3	2610
L.V.	8.5	3.9	1440
L.D.	7.0	2.1	1200
R.J.	8.5	3.7	1370
M.S.	9.0	2.9	1380
E.N.	7.2	2.3	930
E.L.	7.8	3.3	1260
R.T.	7.3	3.0	1080
F.Ma.	6.9	4.4	1200
A.T.	7.0	4.3	840
E.Mo.	6.9	2.4	1260
E.Mi.	8.2	3.1	1230
P.P.	9.2	3.4	2250
R.C.	6.7	3.7	1880
F.Mi.	7.2	3.2	1980
Mean	7.7± 1.1*	3.3±0.6	1560
Range	6.1–10.4	2.1–4.4	(52 days)
Testosterone propionate, 300 mc. weekly, i.m.			
A.B.	4.3	2.3	3600
P.M.	7.4	5.7	1800
S.N.	7.6	4.1	3000
E.P.	9.2	3.1	8100
A.J.	8.8	4.7	600
J.L.	7.2	4.9	1800
M.C.	6.2	5.2	4100
N.B.	8.2	6.4	1400
A.Mo.	6.1	5.0	3200
Mean	7.2±1.4	4.6±1.2	3067
Range	4.3–9.2	2.3–6.4	(71 days)

* Mean and standard deviation.

TABLE 3. THYROXINE-BINDING PROTEIN AND PBI LEVELS IN 3 PATIENTS TREATED WITH 17α-METHYL-19-NORTESTOSTERONE (30 MG. DAILY, BY MOUTH)

Patient	Total dose of 17-MNT (mg.)	Serum PBI (μg./100 ml.)	TBP* (μg./ml.)
F.Mi.	none	7.2	.370
	1410	3.5	.089
R.C.	none	5.9	.330
	1020	3.9	.067
P.P.	none	8.3	.610
	2040	3.4	.063
Control		4.8	.260

* Determinations performed by Dr. J. Robbins.

Steroids in urine and plasma

The mean 24-hour excretion of urinary 17-ketosteroids was studied in 12 of the 20 patients treated with 17-MNT. It fell significantly in all 12. The values for each of these patients are presented in Table 4. The mean 24-

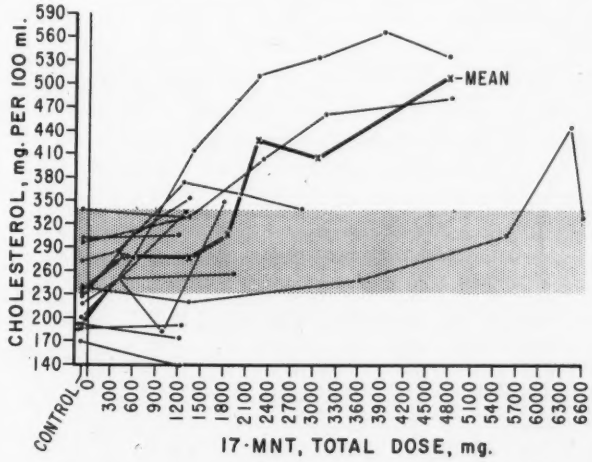

FIG. 2. Levels of serum cholesterol in 13 women prior to, and during treatment with 17α-methyl-19-nortestosterone (30 mg. daily, by mouth). The lower margin of the shaded area represents the mean control level of cholesterol, and the upper margin delimits two standard deviations above the mean. The heavy solid line represents the mean levels of cholesterol with the cumulative dose indicated.

TABLE 4. URINARY EXCRETION OF GONADOTROPINS AND STEROIDS IN PATIENTS WITH METASTATIC CARCINOMA OF THE BREAST BEFORE AND AFTER THERAPY WITH 17α-METHYL-19-NORTESTOSTERONE OR TESTOSTERONE PROPIONATE

Subject	Gonadotropins (% of control rat ovarian wt.)		17-Hydroxycorticosteroids (mg./24 hrs.)		17-Ketosteroids (mg./24 hrs.)	
	Pre-therapy	Post-therapy	Pre-therapy	Post-therapy	Pre-therapy	Post-therapy
17α-Methyl-19-nortestosterone, 30 mg. daily, by mouth						
J.B.	586	72	6.8	2.0	10.1	6.3
A.T.	609	79	2.6	1.6	3.3	3.0
M.R.	773	95	0.6	0.7		
E.Mo.	846	82	1.5	1.4	5.6	2.6
E.Mi.	1548	77	5.7	1.2	8.1	4.2
P.P.	492	76	6.3	1.2	5.6	3.9
R.C.	743	61	5.9	2.4		
F.Mi.	309	113	4.4	2.9		
F.Ma.			2.2	2.4	3.6	2.8
F.R.			4.8	0.7	12.4	5.2
L.V.			4.9	2.6	15.9	4.8
L.D.			4.0	1.9	7.6	3.4
R.J.			2.0	1.6	11.9	6.6
M.S.			4.1	2.9	4.7	2.4
E.L.			12.8	4.7	11.6	5.2
Mean	738	80	4.6±2.8*	2.0±1.0	8.5±3.8	4.2±1.4
P value**			<0.001		<0.001	
Testosterone propionate, 300 mg. weekly, i.m.						
A.B.	47	77	0.7	0.7		
P.M.	503	93				
S.N.	503	74	3.3	1.5	2.6	13.7
E.P.	449	85			13.7	17.2
A.J.	759	84	4.7	3.3	3.1	18.7
J.L.	858	81	6.4	3.6	7.6	30.3
N.B.	474	600	6.3	0.5		
M.C.	682	93	4.8	6.9	6.7	23.2
A.Mo.	114	92				
A.Mu.			2.1	2.4	8.2	16.4
L.G.			2.9	4.9		
Mean	488	142	3.9±1.9	3.0±2.0	7.0±3.7	19.9±5.4
P value			not significant		<0.01	

* Mean and standard deviation.

** P determined by Student's *t* test.

hour excretion of urinary 17-ketosteroids rose significantly in 6 patients treated with TP in whom this determination was made (Table 4).

The mean 24-hour urinary excretion of 17-hydroxycorticosteroids was significantly reduced in the 15 patients of the 17-MNT group (Table 4), whereas excretion in 8 of the 11 patients given TP was not altered significantly (Table 4).

Plasma 17-hydroxycorticosteroid concentration was unchanged in 8 of the patients receiving 17-MNT. The mean control value was 20 ± 6.3 μg. per 100 ml., and the mean treatment value was 21 ± 9.0 μg. per 100 ml. There was an increase in 4 patients and a decrease in 4. Plasma 17-hydroxycorticosteroid levels decreased in 3 patients and increased in 1 during treatment with TP. The mean control value was 25 ± 6.4 μg. per 100 ml., and the mean treatment value was 17 ± 4.5 μg. per 100 ml. This change was of uncertain statistical significance ($.01 < P < .02$).

Urinary gonadotropins

Quantitative determination of 24-hour urinary gonadotropins was carried out in 8 patients treated with 17-MNT (Table 4). The method of Albert was employed (10). A log-dose response was obtained using pooled postmenopausal urine. The slope observed in our laboratory was significantly different from that observed by Albert (15). Therefore the results were expressed in terms of relative ovarian weights as compared to those of saline-injected control rats. In all 8 patients studied, the excretion of gonadotropins was elevated prior to treatment, but fell to unmeasurable levels and remained suppressed throughout the course of therapy. This suppressing effect was observed as early as twelve days after institution of 17-MNT.

Similar studies were performed in 9 of the patients receiving TP (Table 4). In 2, pretreatment levels of gonadotropins were not elevated and remained unchanged during therapy. In 7 patients, excretion was elevated prior to treatment. In 1 of these 7 (N.B.) there was no alteration in excretion of gonadotropins after twelve days of treatment. In the remaining 6 subjects, treated thirty-eight days or longer, excretion fell below the control values.

Liver function studies

Levels of serum bilirubin were followed in 11 of the 20 patients receiving 17-MNT. The values are shown in Figure 3. In all cases, the levels prior to therapy were within the normal range. None of the subjects had clinical evidence of hepatic metastases. Serum bilirubin concentration rose to abnormal values (more than 1.0 mg. per 100 ml.) in 10 of the 11 patients, the mean peak level being 3.2 mg. per 100 ml. These patients had received

treatment with 17-MNT for seven to thirty-two weeks when the abnormal values were noted; therapy was promptly discontinued. At the end of six weeks of treatment in 1 patient, the level of serum bilirubin was 0.1 mg. per 100 ml. In 5 patients, the level was still elevated two weeks after cessation of therapy. Values returned to normal in 4 women within four to six weeks. In 1 patient , hyperbilirubinemia continued at a level of 1.2 mg. per 100 ml. six weeks after discontinuance of 17-MNT, and only later did the concentration return to normal.

Alterations in serum cholesterol concentration have been discussed earlier.

In 3 women of the 17-MNT group, levels of serum alkaline phosphatase

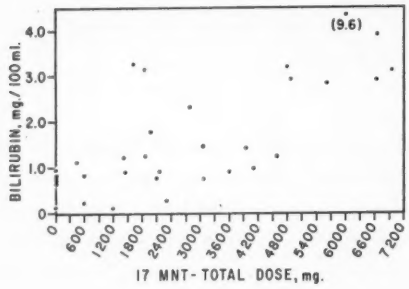

FIG. 3. Levels of serum bilirubin determined serially for from seven to thirty-two weeks in 11 women with progressive metastatic breast carcinoma receiving 17α-methyl-19-nortestosterone (30 mg. daily, by mouth).

were elevated initially to an average of 9.5 Bodansky units (B.U.) per 100 ml. All had radiographic evidence of osseous metastases. During subsequent therapy with 17-MNT, the concentration decreased to an average of 5.9 B.U. per 100 ml. In 13 patients, initial alkaline phosphatase levels were normal; during therapy they were decreased in 7, elevated in 4, and unchanged in 2. One of the elevated values was abnormally high.

In 8 of the 20 patients who later received TP therapy, control levels of serum alkaline phosphatase were elevated. During treatment with TP, values were decreased in 4, unchanged in 2, elevated in 1, and transiently elevated only to be more subsequently decreased in 1. In 12 patients of the TP group, the control levels of serum alkaline phosphatase were normal. No change occurred with treatment in 6; in 4 there was a rise to abnormal levels; and in 2 there was a decrease.

In 5 patients studied (4 of whom became jaundiced) values for albumin and globulin during therapy with 17-MNT remained normal. In 1, hyperglobulinemia occurred during treatment. In 3 patients the thymol turbidity value was normal at the time they became jaundiced while receiving 17-MNT; the thymol turbidity value was elevated in the patient with hyperglobulinemia. In 3 patients, the plasma prothrombin time was normal at

the time jaundice was noted. Excretion of urinary urobilinogen was normal in 2 jaundiced patients. Serial studies of liver function in 1 representative patient during therapy with 17-MNT are shown in Figure 4.

Chemical and hematologic studies

Serial determinations of the 24-hour urinary excretion of calcium, phosphorus, citric acid and creatinine were carried out before and after treatment, in 13 patients treated with 17-MNT and in 10 patients treated with TP. With 1 exception, all were ambulatory and receiving unrestricted diets. There were no major alterations in these urinary constituents during therapy. The range of values was wide for all parameters.

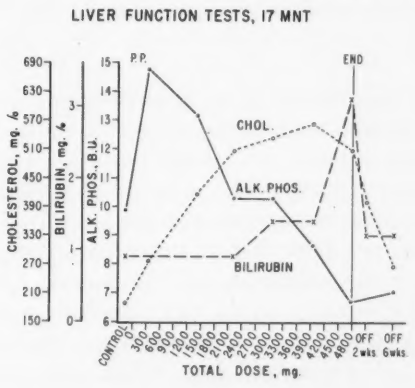

FIG. 4. Levels of serum bilirubin, cholesterol and alkaline phosphatase determined serially in the patient described in the legend to Figure 1.

No change was noted in values for blood urea nitrogen, fasting blood sugar, serum sodium or serum phosphorus in patients receving either 17-MNT or TP. In 1 patient receiving TP, the serum potassium level fell to 2.5 mEq. per liter, but no significant change was noted in the other patients during treatment with either drug. Hypercalcemia developed in 1 of 20 women receiving TP, but serum calcium levels remained within the normal range in all the other subjects receiving TP and in all receiving 17-MNT.

In 8 of the subjects treated with 17-MNT, serum citric acid concentration decreased in 7 and was unaltered in 1. The mean control level of serum citric acid was 3.2 ± 0.9 mg. per 100 ml. and the mean value during therapy was 1.9 ± 0.4 mg. per 100 ml. ($P < .01$). Levels of serum citric acid in 7 of the patients receiving TP were decreased in 3, increased in 2, and unchanged in 2. The mean control level was 2.8 ± 1.5 mg. per 100 ml. and the mean value during TP therapy was 2.3 ± 0.4 mg. per 100 ml. This difference was not

statistically significant ($P < 0.4$). The level of serum calcium in all these patients was within the normal range.

Blood hemoglobin concentration was determined serially in 17 of the patients receiving 17-MNT. There were no instances of polycythemia. In 1 patient there was a significant increase, in 2 there was a decrease and in 14, no change. Levels of hemoglobin were determined serially in 18 of the subjects given TP; in 5 there was a significant rise to polycythemic levels. The mean control hemoglobin value was 12.6 Gm. per 100 ml. with a range of 11.9 to 14.7 Gm. per 100 ml., and the mean peak value after three to ten months of TP therapy was 16.6 Gm. per 100 ml. with a range of 14.8 to 19.6 Gm. per 100 ml. In 10 patients there was no alteration in the level of hemoglobin during treatment, and in 3 women there was a decrease (associated with hemorrhage in 1).

DISCUSSION

17α-Methyl-19-nortestosterone (17-MNT) is one of a series of 19-norsteroids with potent progestational activity, as determined by various biologic assays in female rabbits, rats and humans (16–23). Its anabolic (myotropic) activity in rats is comparable to that of testosterone propionate or methyltestosterone (18, 23). Its androgenic potency in rats is 20 to 50 per cent that of testosterone propionate (23), whereas the estrogenic effect is negligible (19). In women, 17-MNT has been used therapeutically for disturbances of the menstrual cycle and as an oral contraceptive (22). Adverse effects were not noted when doses of 1 to 5 mg. were administered daily for several days. When doses of 5 to 50 mg. were given daily for several weeks hirsutism, deepening of the voice, nausea and rapid gain in weight were noted only rarely (24).

In the present study, with a dosage of 30 mg. daily for long periods of time, androgenicity was marked in 7 of 20 women. The effects were similar to those induced by testosterone propionate (TP). The gain in weight that occurred in the majority of the women was due to retention of fluid rather than to any anabolic effect, since it was accompanied by edema and was reversed by diuretic therapy. In contrast to the results of animal assays, the fact that withdrawal uterine bleeding occurred in 40 per cent of these castrated or postmenopausal women indicates the estrogenic activity of 17-MNT when administered orally in large doses.

It has been observed (25) that in male subjects given 25 mg. of 17-MNT daily there is a reduction in the 24-hour urinary excretion of 17-ketosteroids with no change in the excretion of 17-hydroxycorticosteroids. In the present study, the reduction in urinary excretion of both 17-ketosteroids and 17-hydroxycorticosteroids might be explained by suppression of adrenocortical activity, mediated through the anterior pituitary, but no studies were

performed to test this hypothesis. Administration of TP did not result in alteration of the urinary excretion of 17-hydroxycorticosteroids (*cf.* (26)), nor did it have any significant effect on the level of plasma 17-hydroxycorticosteroids (*cf.* (27)).

17-MNT proved a potent agent in suppressing urinary gonadotropins; there was an early and absolute decrease in excretion. This effect was more constant than that obtained with TP.

Alterations in the levels of serum protein-bound iodine (PBI) and thyroxine-binding protein (TBP), in the absence of signs or symptoms of thyroid dysfunction, have been observed during administration of estrogens (28, 29) or androgens (30). In our study, the decrease in the levels of PBI and TBP in subjects given 17-MNT was similar to that observed by others during administration of methyltestosterone (31). The effect was more marked than that associated with the administration of TP, as also noted in other reports (30).

The action of 17-MNT on these parameters has been studied by us in greater detail in the course of administering the drug (30 mg. daily by mouth for two weeks) to a 36-year-old woman in whom the menopause had occurred two years previously (15). In this subject, the serum PBI level fell by the second day of treatment and continued to fall throughout therapy. The control level was 5.8 μg. per 100 ml., and the value on day 14 of treatment was 3.7 μg. per 100 ml. The fall in TBP was detectable on the fourth day of treatment, and there was a further reduction to one-third of the control value by day 14. Elevation of the BMR was apparent on the tenth day of treatment, although the serum level of free thyroxine was unchanged.

In contrast to the experience of Keitel and Sherer with methyltestosterone (30), no decrease in the thyroidal uptake of I^{131} was observed in patients given 17-MNT. Clinically, they showed no evidence of thyroid dysfunction.

The mechanism of these effects is not clear. Studies of thyroxine disappearance rates, thyroidal iodide clearance, iodine pools and iodine degradation are being undertaken in order to differentiate the direct effects of 17-MNT on the thyroid from extrathyroidal mechanisms. Similar studies by Federman *et al.* in patients given methyltestosterone for four weeks have demonstrated no change in iodide metabolism, but an increase in the thyroxine disappearance rate and an increase in the absolute degradation of hormonal iodine (31). It was suggested that the primary effect of methyltestosterone was the production of a profound decrease in the concentration of TBP in serum. Resultant changes in thyroid function included an elevated level of free thyroxine, increased thyroxine metabolism, and suppression of thyrotropin with decreased thyroxine secretion leading to a fall

in serum PBI concentration. With achievement of a steady state, normal values would be obtained for thyroxine, thyroidal iodide clearance, thyroidal hormone secretion and thyroxine degradation (31).

Studies by Engbring and Engstrom (32) in patients give testosterone propionate for two to three weeks showed no change in thyroxine survival time nor any consistent change in TBP, although levels of PBI decreased significantly.

The occurrence of marked hypercholesteremia during long-term therapy with 17-MNT does not appear to be related to hypothyroidism, since there was a difference between the time sequences of the effects on thyroid function and on the level of cholesterol. No change in serum lipid concentration was noted in the early phases of therapy; hypercholesteremia developed at eight weeks, and peak values at three months. Similar rises in the level of serum cholesterol, though of lesser degree, have been observed during administration of methyltestosterone (31). A decrease in the level of serum alpha-lipoprotein (S_f0-12) has been reported in male patients receiving 17-MNT (33). Whether the effects are upon cholesterol or lipoprotein synthesis or excretion, or whether they represent a hepato-toxic manifestation of prolonged drug therapy, is not clear. Similar results were not observed in subjects receiving TP. This suggests a dissociation of androgenicity *per se* from the cholesterol-elevating potential of 17-MNT. The hypercholesteremic effect of 17-MNT may also be related to the oral route of administration, in contrast to the parenteral route for TP. Results are at variance with the cholesterol-lowering effect of androsterone (34).

The occurrence of jaundice in patients receiving 17-MNT has been reported previously by Peters *et al.* (35) in 2 male patients who had received a total dose of 1,378 mg. and 12,600 mg. respectively. Pathologic findings in the liver were similar to those observed in cases of jaundice developing in the course of treatment with methyltestosterone. The laboratory findings were those of acute biliary obstruction (35). Intrahepatic cholestasis was reported by Schaffner *et al.* (36) in 4 of 27 subjects receiving a related steroid, 17-ethyl-19-nortestosterone (Nilevar), in a dosage of 60 mg. daily for three to five weeks. Severe jaundice developed in 1 patient. Most cases of drug-induced cholestasis were associated with increased activity of serum alkaline phosphatase. This was not invariable in the subjects treated with Nilevar. The lack of correlation with biochemical findings suggested that the pathologic process was neither inflammatory nor allergic, but was perhaps due to altered permeability of bile ductules or increased viscosity of the bile. Abnormal bromsulphalein (BSP) retention, changes in the serum glutamic oxalacetic transaminase content, and elevation of the serum bilirubin level have been reported with great frequency in patients treated with Nilevar (37). There was a direct relationship to the dosage, and reversal of

chemical abnormalities occurred after discontinuation of the drug. Heany and Whedon (38) found that alterations in BSP removal, without changes in the results of other routine liver function tests, occurred within seven to ten days in normal subjects given Nilevar or methyltestosterone, but not in subjects given testosterone propionate. Jaundice has occurred only rarely in patients given methyltestosterone.

Elevation of the serum bilirubin content occurred in all patients receiving more than 4,200 mg. of 17-MNT in the present study. This was not correlated with any uniform change in the results of routine liver function tests, other than the association with hypercholesteremia. BSP removal was not determined in these patients, since control values in several subjects were abnormal. In 1 healthy control subject given 17-MNT for two weeks, however, BSP retention rose from a control value of 0 per cent to 33 per cent at the termination of treatment (15).

The data suggest: 1) that 17-MNT has the same propensity as its related 17-alkyl testosterone derivatives for inducing early retention of BSP, 2) that its administration is associated with a greater incidence of jaundice related to drug dosage, and 3) that it does not alter the findings in most routine tests of liver function, but has a singular hypercholesteremic effect and probably causes intrahepatic cholestasis. The combination of cumulative effects seriously limits the long-term use of this agent.

Extensive studies of the chemical constituents of urine and blood failed to reveal any major alterations in most parameters. The low incidence of hypercalcemia and hypokaliemia in the group of patients receiving TP is noteworthy. There was no significant alteration in the levels of serum electrolytes in patients receiving 17-MNT despite the frequency of retention of fluid and edema. The depression of serum citric acid levels in the subjects receiving 17-MNT is of interest. Other investigators have reported similar changes in serum citric acid concentration in patients receiving 17-hydroxycorticosteroids in whom the levels of plasma steroids are increased (39). Testosterone propionate and estradiol benzoate given parenterally have failed to effect any change in serum citric acid concentration (40).

Earlier studies with methyltestosterone and TP administered to eunuchs demonstrated a rise in the concentration of hemoglobin (41). This effect was prominent (5 of 18 patients) in a group of women with carcinoma of the breast treated with TP, but was negligible (1 of 17 patients) in cancer patients receiving 17-MNT.

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STUDIES ON THE BIOLOGIC CHARACTERIZATION OF HUMAN GONADOTROPINS. IV. COMPARISON WITH TWO NEW PREPARATIONS OF HPG FROM THE URINE OF POST-MENOPAUSAL WOMEN*

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ABSTRACT

Two new preparations of human pituitary gonadotropin (HPG) from postmenopausal urine obtained by carbunite adsorption and by kaolin-zeolite adsorption, respectively, were tested in the assay system employing the simultaneous response of the ovary and uterus in the immature rat. The parameters of these assays then were compared with similar parameters obtained from HPG made by the kaolin-acetone method. The constants of the assays for HPG from postmenopausal urine prepared by these 3 different methods did not differ.

OUR previous studies of human gonadotropins have shown a close similarity of human pituitary gonadotropin (HPG) from the urine of postmenopausal or castrated women when prepared by different extraction methods (1). Specifically, HPG prepared by the kaolin-acetone method or by the ultrafiltration method gave the same constants in a biologic assay system utilizing the simultaneous response of the ovary and uterus of intact rats. Furthermore, the same constants were obtained when HPG was prepared from the urine of men by the kaolin-acetone process and further purified by precipitation with alcohol.

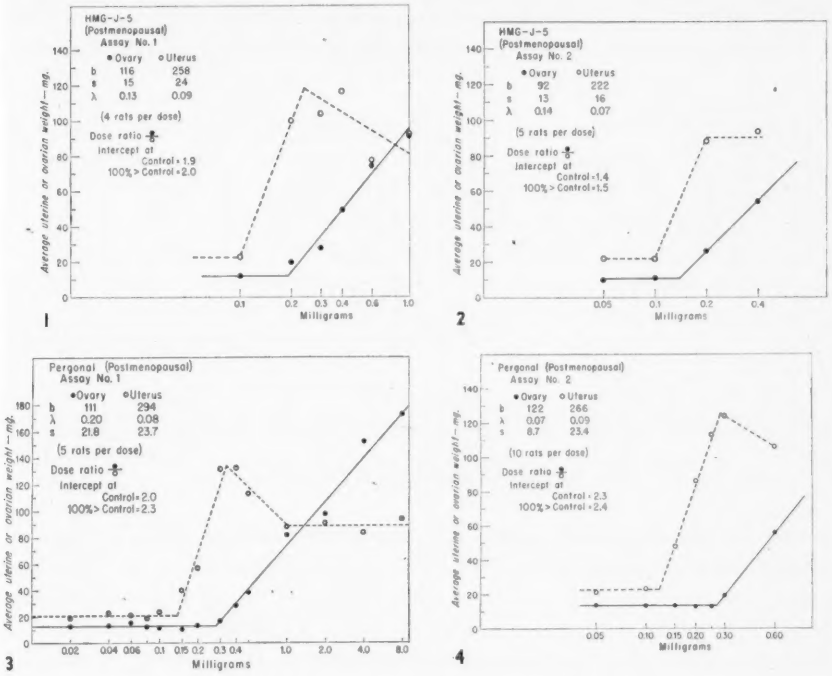
The purpose of this report is to extend these observations to two new preparations of HPG obtained from the urine of postmenopausal women. The first of these (HMG-J-5) was made by Dr. Svend Johnsen by the use of carbunite adsorption. The second was made by Dr. P. Donini by means of a combined adsorption method using kaolin and zeolite; it is termed "Pergonal." Both preparations have a high specific activity. The methods for their preparation differ considerably from those used in our previous studies. Because it is possible that the biologic composition of HPG may differ as a result of the method of preparation, it was deemed desirable to investigate the biologic properties of these two new HPG materials by the same system utilized in our previous studies.

Received January 23, 1960.

* Aided by Grant A-1738, National Institutes of Health, United States Public Health Service.

MATERIAL AND METHODS

Sufficient quantities of HMG-J-5 and Pergonal to carry out two assays were generously furnished by Dr. Johnsen and Dr. Donini. Preliminary assay of these materials according to the routine method previously described (2) showed that HMG-J-5 contained 23.6 rat units per milligram, whereas Pergonal contained 18.1 rat units per milligram. Suitable dilutions were made to embrace the response of both the uterus and ovary in the same animals, and two assays of each preparation were completed. The details of this assay method and the method for constructing composite curves in absolute coordinates have been described (1, 3).



FIGS. 1–4. Assays of HPG preparations.

RESULTS

Figures 1 and 2 show respectively a six-dose and a four-dose assay using HMG-J-5. The two assays agree closely. Furthermore, the slopes of both the ovarian and the uterine response and the dose ratios at the control level are the same as those observed in a series of similar assays of HPG from postmenopausal women after preparation of the urine by our kaolin-acetone method. Figure 3 represents a fourteen-dose assay and Figure 4 a seven-dose assay using Pergonal. Both assays show close agreement, and

again the parameters of the assay are not different from those previously described for postmenopausal HPG prepared by our kaolin-acetone method. Composites of the assays for each substance are shown in Figure 5, which is plotted in absolute coordinates. The types of curves and the constants of the dose-response lines are the same.

When the respective slopes of the dose-response curves for two gonadotropins are identical, the dose ratios of the ovarian and uterine intercepts at control values possibly may give evidence of any dissimilarity of a biologic nature. In the previously reported eight assays of postmenopausal HPG prepared by the kaolin-acetone method, the dose ratio ranged from 1.4 to 2.0. The mean was 1.6, with a standard deviation of 0.2. The dose

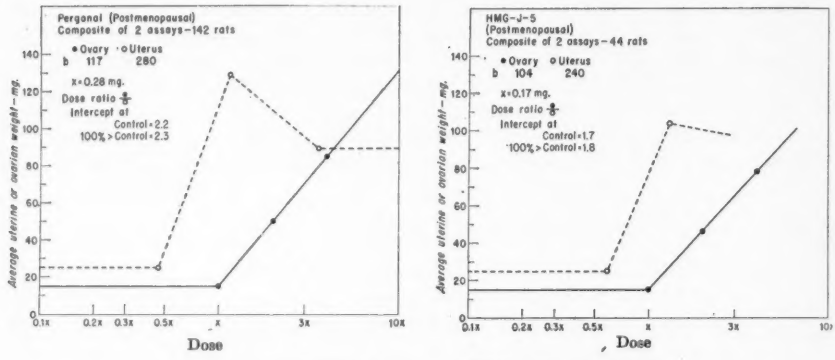


FIG. 5. Composite assay of HPG preparation.

ratios for the two assays using HMG-J-5 were 1.4 and 1.9; for the two assays using Pergonal, they were 2.3 and 2.0. These values are within or close to three standard deviations of the mean (1.0–2.2) of the eight dose ratios previously determined. Thus, as Table 1 indicates, no difference in biologic nature can be detected in postmenopausal HPG when prepared by these 3 different methods of extraction and purification.

Because of its high potency, postmenopausal urine appears to be the most likely source of material for the preparation of an international reference standard of HPG. The establishment of a standard requires a great deal of urine, and large-scale processing of urine may necessitate the use of extraction methods different from the kaolin-acetone procedure commonly employed for clinical specimens (24-hour or 48-hour specimens of urine, or 1.5 to 3.0 liters). Therefore, the data reported here are reassuring in that HPG prepared by 2 large-scale extraction methods (HMG-J-5 and Pergonal) appears to be the same as that prepared by our routine kaolin-acetone extraction method for clinical specimens. This indicates that clin-

TABLE 1. CHARACTERIZATION OF 3 PREPARATIONS OF HUMAN PITUITARY GONADOTROPIN FROM POSTMENOPAUSAL URINE BY PARAMETERS OF DOSE-RESPONSE CURVES

Preparation	No. of assays	Ovary		Uterus		Mean dose ratios, intercepts at control
		$\bar{b}$*	$\bar{\lambda}$†	$\bar{b}$*	$\bar{\lambda}$†	
Kaolin-acetone (K.A.)	8	113	0.12	239	0.14	1.6
Carbunite (HMG-J-5)	2	104	0.14	240	0.08	1.7
Kaolin-zeolite (Pergonal)	2	117	0.14	280	0.09	2.2

* $\bar{b}$ = Mean slope.
† $\bar{\lambda}$ = Mean index of precision.

ical assays may be conducted in conjunction with a standard obtained by a different procedure, at least in the case of the foregoing two procedures. Such assurance, however, would be valid only when the assay was carried out by a general gonadotropin assay like the rat ovarian-weight technic because, even if the 3 HPG materials were found to differ biologically by other technics, such differences apparently are not reflected in the rat-ovary assay. Fortunately, this assay is in wide use, at least in this country, for clinical purposes.

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TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA WITH CONCENTRATED HYDROCORTISONE ACETATE

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ABSTRACT

Concentrated hydrocortisone acetate (200 mg. per ml. of solution) was administered intramuscularly at two-week intervals to 10 patients with congenital adrenal hyperplasia ranging in age from 27 months to 9 years 10 months. The results at the end of a six- to nine-month study period were as follows: 1) the virilization and excessive urinary excretion of androgens were adequately suppressed in all cases, 2) precocious bony maturation and linear growth were arrested in all cases, 3) urinary 17-ketosteroid excretion remained suppressed between injections, without fluctuations, and 4) the occurrence of large subcutaneous sterile abcesses in 3 children proved a major deterrent to the use of this preparation.

THE corticosteroids available at present for administration by the parenteral route require frequent injection to obtain satisfactory biochemical and clinical responses in the treatment of patients with congenital adrenal hyperplasia. In 1955 Segaloff, Gordon and Horwitt (1) reported on the successful use of hydrocortisone acetate administered intramuscularly at intervals of one to three weeks. The large volume of the injected material (250 mg. suspended in 10 ml. of physiologic sodium chloride) prohibits its use in younger children. Adequate suppression of abnormal steroidogenesis has also been reported by Prader (2) using macrocrystalline cortisone (800 mg. injected every thirty days). Another long-acting injectable steroid, a mixture of cortisone enanthate and cortisone undecylinate, elicited satisfactory response in a patient treated by Skeel (3). Despite these favorable reports, the use of long-acting steroids administered infrequently has been rejected, mainly on the theoretical grounds that uniform suppression of virilization cannot be expected (4).

The purpose of the present communication is to report the results of treatment in 10 cases of congenital adrenal hyperplasia, using a concentrated form of hydrocortisone acetate intramuscularly every two weeks.

Received May 19, 1959.

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MATERIALS AND METHODS

Nine patients with congenital adrenal hyperplasia, ranging in age from 2 to 10 years, were taken at random from the Endocrine Clinic of the Children's Hospital, Los Angeles. An additional subject included in this report will be described separately from the study group because: 1) her case provided the initial stimulus for the present investigation, and 2) she had been treated with infrequent injections (every two to three weeks) of hydrocortisone for a longer period than had the other 9 subjects.

Baseline determinations of bone age, and of the 24-hour urinary excretions of 17-ketosteroids (17-KS)[1] and pregnanetriol were obtained at the beginning and end of the study. Urinary 17-KS were measured by the method of Drekter *et al.* (5) and pregnanetriol by the method of Cox (6).

Bone age was estimated by two standards: 1) the Atlas of Greulich, Pyle and Todd (7) utilizing roentgenograms of the hand and wrist, and 2) unilateral roentgenograms of the shoulder, wrist, hand, hip, knee and foot as described by Wilkins (4).

The two weeks preceding the first injection of concentrated hydrocortisone acetate (F.Ac.) constituted the control period. All of the patients were receiving glucosteroids (compound E or F, with or without DCA) and no change was made in their therapy during this period. The initial dose of F.Ac.[1] was 200 mg. (1 ml.), intramuscularly, or an amount equal to the total of the other steroids given orally or by injection during the two-week control period. Urine (24-hour specimens) for 17-KS determinations was collected on the seventh and fourteenth days after the injection. F.Ac. was given every two weeks and the dose was regulated according to the level of 17-KS excretion.

RESULTS

The subjects were divided into two groups, salt-losers and non-salt-losers. Table 1 summarizes the status of the 6 salt-losers during the control period. The column "total two-week dose" represents the amount of exogenous steroid each child received during the control period. Desoxycorti-

TABLE 1. SALT-LOSERS: STATUS BEFORE TREATMENT WITH CONCENTRATED F ACETATE—CONTROL PERIOD

Patient	Sex	Age	Bone age	Height age*	Urinary 17-KS (mg./24 hrs.)	Urinary pregnanetriol (mg./24 hrs.)	Steroid dosage (mg.)	Total 2-week dose (mg.)	DCA admin'd
1	♀	6 yrs. 10 mos.	12 yrs.	9 yrs. 8 mos.	11.5		E 50 i.m. t.i.w.†	300	
2	♂	6 yrs. 9 mos.	11–12 yrs.	7 yrs. 8 mos.	4.6	7.3	E 50 i.m. q.o.d.‡	350	+
3	♀	2 yrs. 3 mos.	2 yrs. 6 mos.	2 yrs.	3.8		F 10 p.o. daily	140	+
4	♀	3 yrs. 7 mos.	4 yrs. 2 mos.	3 yrs. 6 mos.	0.9	0.9	F 15 p.o. daily	210	+
5	♂	7 yrs. 6 mos.	13 yrs. 3 mos.	9 yrs. 6 mos.	5.4	2.7	F 50 i.m. daily	700	
6	♂	8 yrs.	14 yrs.	12 yrs. 6 mos.	5.9		E 50 i.m. 5× wk.	500	

* Watson & Lowrey.
† Three times weekly.
‡ Every other day.

[1] Kindly supplied by the Upjohn Co., Kalamazoo, Michigan, as Cortef Acetate, 200 mg. per ml.

TABLE 2. NON-SALT-LOSERS: STATUS BEFORE TREATMENT WITH CONCENTRATED F ACETATE

Patient	Sex	Age	Bone age	Height age	Urinary 17-KS (mg./24 hrs.)	Urinary Pregnanetriol (mg./24 hrs.)	Steroid dosage (mg./24 hrs.)	Total 2-week dose (mg.)
7	♀	6 yrs. 10 mos.	8 yrs. 10 mos.	10 yrs.	0.9		E 35 p.o.	490
8	♀	6 yrs. 2 mos.	6 yrs. 10 mos.	5 yrs.	1.5		F 15 p.o.	210
9	♀	9 yrs. 10 mos.	15 yrs.	12 yrs. 6 mos.	4.7	4.8	F 30 p.o.	420

costerone acetate (DCA) was discontinued after F.Ac. was started in Cases 3 and 4.

In Table 2 similar data are presented for the 3 non-salt-losers during the control period. All were receiving oral steroid therapy at this time.

Effects of F.Ac.

The results at the end of the six- to nine-month study period are shown in Table 3. The urinary steroid values listed in this table were for urine samples collected two weeks after the last injection of F.Ac. It is apparent that adequate suppression of both 17-KS and pregnanetriol was achieved in each child. In 7 children this suppression was greater than that during the control period. In 5 of the 7, the dose of exogenous steroid was less than that given during the control period.

Skeletal maturation was not significantly altered during the study period. The rate of linear growth, using Watson and Lowrey's standards (8), was substandard in the majority of subjects. The inherent inaccuracies in measurements of height, the relatively short duration of the study period, and the small number of subjects limit the significance of this observation.

The 24-hour excretion of 17-KS was determined over a two-week period

TABLE 3. RESULTS AT END OF STUDY

Patient	Age	Length of study (months)	Urinary 17-KS (mg./24 hrs.)	Change in 17-KS (mg./24 hrs.)	Urinary pregnanetriol (mg./24 hrs.)	Dosage of F.Ac. (mg.q.2 wks.)	Change in 2-week dose (mg.)	Change in bone age	Increase in height (inches)
1	7 yrs. 4 mos.	6	4.7	−6.8	1.7	400	+100	Minimal	1½
2	7 yrs. 6 mos.	9	1.2	−3.4	0.7	300	− 50	Minimal	1
3	2 yrs. 11 mos.	8	0.8	−3.0	0.5	300	+160	+6 mos.	2
4	4 yrs. 1 mos.	6	1.2	+0.3	1.0	300	+ 90	+8 mos.	1½
5	8 yrs. 2 mos.	6	3.2	−2.2	2.6	300	−400	No chg.	½
6	8 yrs. 8 mos.	8	5.7	−0.2	2.9	400	−100	Minimal	1
7	7 yrs. 5 mos.	7	3.0	+2.1	1.0	320	−170	Minimal	1
8	6 yrs. 8 mos.	6	0.9	−0.6	0.6	200	− 10	No chg.	½
9	10 yrs. 6 mos.	8	1.8	−2.9	0.5	280	−140	Minimal	½

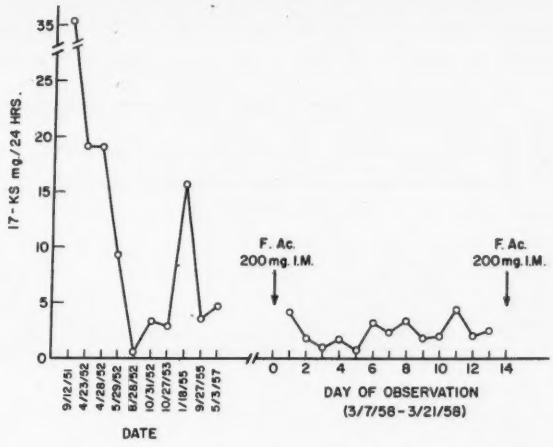

FIG. 1. Suppressive effect of steroid therapy on 17-ketosteroid excretion in Patient #9, during the control period and between injections during the F.Ac. period.

between injections in order to verify the impression that virilization was adequately suppressed with F.Ac. Figure 1 demonstrates the validity of this impression. The 24-hour excretion of 17-KS in this 10-year-old girl did not exceed 5 mg. during this two-week period.

The time required for optimum adjustment to F.Ac. varied from four to thirteen weeks.

Complications

Five sterile abscesses occurred in 3 children. Two required surgical drainage and the others drained spontaneously. The majority of the 160 intramuscular injections during the study period were given by the treatment nurse; all abscesses occurred following injections given by the children's parents. It seemed likely that they resulted from inadvertent subcutaneous injection of the steroid. In spite of these untoward reactions, the children who had received steroids intramuscularly prior to the investigation preferred the new preparation. This, no doubt, was related to the decreased frequency of injections. With F.Ac., pain at the site of injection was no greater than that associated with the previous intramuscular medication.

Case report

Patient No. 10. This 5-year-old girl was born on February 28, 1953. The diagnosis of congenital adrenal hyperplasia had been made at 11½ months of age, and treatment had been started. Incomplete suppression of virilization resulted while she was receiving oral steroids. Intramuscular hydrocortisone (50 mg. per ml.) had been started when she was

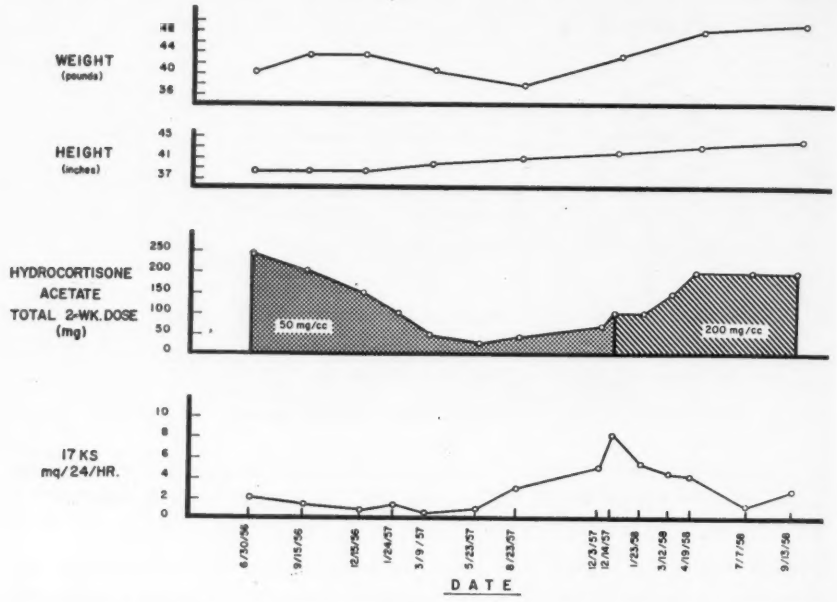

FIG. 2. Effect of steroid therapy on weight, height and 17-ketosteroid excretion in Patient #10. The stippled area represents treatment with hydrocortisone intramuscularly (50 mg. per injection at varying intervals); the cross-hatched area represents treatment with concentrated hydrocortisone acetate (stabilized at 200 mg. every two weeks).

26 months old. She first came under our care in June 1956, when she was three years old. At that time (Fig. 2) she was receiving hydrocortisone, 50 mg. intramuscularly twice a week. Evidence of excessive steroid dosage was manifested by inhibition of linear growth and rounding of the face; this necessitated decreasing the amount and frequency of injections. From March to August, 1957, adequate control was achieved with only 50 mg. every two to three weeks. Thereafter, "escape" occurred during treatment with this small amount of steroid. In December 1957, therapy with concentrated hydrocortisone acetate (F.Ac.) was started. After a period of adjustment, the dosage was stabilized at 200 mg. every two weeks. Five months later (September 1958) the excretion of 17-KS and pregnanetriol, skeletal maturation, linear growth and dental maturation were normal. No untoward local or systemic reactions resulted from the injections.

DISCUSSION

Many adrenal corticosteroids administered orally or parenterally cause a decrease in the abnormal excretion of steroids in previously untreated patients with congenital adrenal hyperplasia (4, 9, 10–13).

The response to therapy, regardless of the steroid used, usually is correlated with the dosage and frequency of administration (9). It is difficult,

however, to equate the dosage to any parameter of body size because of the limited number of cases available and the variability in the degree of virilization of individual patients.

Some patients seem to respond better to intramuscular than to oral therapy. This has been noted especially in infants under two years of age. Another factor to be considered in selecting the route of administration is the frequency of vomiting associated with many illnesses during childhood. When this symptom is present, the need for additional corticosteroid may be urgent. Sudden death has been reported after the onset of stressful illnesses such as measles (14). We have observed 3 deaths in patients receiving oral steroids during febrile illnesses associated with vomiting. Two of these children lived a considerable distance from a medical center. Although the parents had received instruction in intramuscular therapy and had the necessary supplies, parenteral steroids were not given. These deaths might have been avoided if regular intramuscular therapy had been continued.

Acknowledgment

The authors gratefully acknowledge the referral of Case 10 by Dr. Robert Ulstrom, Associate Professor of Pediatrics, University of Minnesota School of Medicine, and the assistance and advice of Dr. Howard A. Joos, Associate Professor of Pediatrics, University of Southern California School of Medicine.

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CEREBROSPINAL FLUID GAMMA GLOBULIN CONCENTRATION IN MYXEDEMA*

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ABSTRACT

The concentrations of gamma globulin and total protein in the cerebrospinal fluid of normal and myxedematous subjects were determined by a modification of the tyrosine equivalence method. In 10 of 14 patients with myxedema, there was an elevation of the gamma globulin/total protein ratio not attributable to serum hypergammaglobulinemia. In 4 of these cases, an elevated concentration of spinal fluid gamma globulin was present despite a normal level of total cerebrospinal fluid protein. These data cast doubt on the hypothesis that permeability of the blood-cerebrospinal fluid barrier to serum proteins accounts for the elevation of spinal fluid protein in myxedema.

THE frequent elevation of the cerebrospinal fluid protein level in patients with myxedema was documented by Thompson and his coworkers in 1928 (1, 2). Recently this phenomenon was restudied by Bronsky *et al.* (3), who confirmed the original findings and presented additional data on the electrophoretic partition of cerebrospinal fluid proteins. These investigators described an increase in the concentration of spinal fluid gamma globulin proportional to the increase in concentration of total protein, and concluded that it could be attributed to loss of integrity of the blood-cerebrospinal fluid barrier.

The present study was undertaken to examine more critically the increase in spinal fluid gamma globulin in myxedema, employing a single chemical method that determines both total protein and gamma globulin concentrations. The results indicate that the level of spinal fluid gamma globulin may be increased in myxedema despite a normal level of total

Received August 20, 1959.

* Supported in part by grants from the United States Public Health Service and the National Multiple Sclerosis Society.

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cerebrospinal fluid protein, and cast doubt on the hypothesis that a simple alteration in permeability is the cause.

MATERIAL AND METHODS

The 14 patients, aged 7 to 58 years, with proven primary myxedema[1] had either never received thyroid replacement therapy, or had had no medication for at least eight weeks. In this group were 4 patients with presumed idiopathic atrophy of the thyroid, 4 with biopsy-proved Hashimoto's struma, 3 siblings with goitrous cretinism, 2 patients with post-surgical myxedema, and 1 sporadic athyreotic cretin. Ten adults without evidence of neurologic or chronic systemic disease served as controls.

Ten-milliter samples of cerebrospinal fluid were obtained by lumbar paracentesis. No specimen contained more than an occasional red or white blood cell. The concentrations of total protein and gamma globulin were determined by a modification of the tyrosine equivalence method reported by Papadopoulos *et al.* (4). Total protein was determined on a 1:5 dilution of cerebrospinal fluid in 1.25 per cent aqueous sodium carbonate. To a 2-ml. sample of this dilution were added 2 ml. of 0.05 per cent copper sulfate aqueous solution, and 5 ml. of 12.5 per cent aqueous sodium carbonate. After incubation at 37° C. for thirty minutes, 1 ml. of phenol reagent (Folin and Ciocalteau (5)) in a dilution of 1:3 was added, and color development allowed to take place at room temperature for fifteen minutes. The reading was made in a Coleman spectrophotometer at 540 mμ, and the protein concentration was calculated from a standard curve obtained with the use of dialyzed dried serum.[2] Gamma globulin was precipitated from the cerebrospinal fluid for fifteen hours in the refrigerator, employing zinc sulfate in barbital buffer at pH 7.3. The precipitate was dissolved in 2 ml. of 1.25 per cent sodium carbonate solution and assayed by the same procedure as for total protein. The final result was calculated from a standard curve obtained with the use of gamma globulin.[3]

Since the foregoing method has not been applied to analysis of serum, partition of serum proteins was studied by hanging-strip paper electrophoresis using barbital buffer at pH 8.6. Protein fractions were stained with aqueous bromphenol blue, and quantitated by means of a Spinco Analytrol densitometer (6). The gamma globulin fraction was expressed directly as a percentage of the total protein.

RESULTS

Control subjects

Data on the 10 control cerebrospinal fluid samples are presented in Table 1. Mean values for total protein and gamma globulin were 37.0 and 3.2 mg. per 100 ml., respectively. The ratio of gamma globulin to total protein ranged from 5.3 to 11.0 per cent, with a mean value of 8.6 per cent.

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[1] Thirteen subjects had serum protein-bound iodine levels of less than 2.5 μg. per 100 ml. The remaining patient, with profound clinical myxedema and Hashimoto's struma proved by biopsy, had a 24-hour thyroidal I^{131} uptake of 14 per cent; the uptake failed to increase after administration of thyrotropin.

[2] Versatol,® Warner Chilcott Laboratories, Morris Plains, New Jersey.

[3] Protein Foundation Laboratories, Jamaica Plain, Massachusetts.

TABLE 1. CEREBROSPINAL FLUID PROTEINS IN 10 CONTROL AND 14 MYXEDEMA PATIENTS

Subject No.	Total protein (mg./100 ml.)	γ-Globulin (mg./100 ml.)	γ-Glob./Tot. prot. (%)
	Control		
1	24.0	2.6	10.8
2	30.0	2.7	9.0
3	35.0	3.5	10.0
4	38.0	2.0	5.3
5	38.0	2.2	5.8
6	38.0	3.1	8.2
7	38.0	4.2	11.0
8	40.0	4.0	10.0
9	44.0	3.7	8.4
10	45.0	3.5	8.2
Mean	37.0	3.2	8.6
Std. Dev.	6.2	0.77	1.9
	Myxedema		
1	20.0	3.5	17.5
2	20.0	5.8	29.0
3	22.5	5.2	23.1
4	26.0	3.0	11.5
5	26.8	5.0	18.6
6	27.5	2.0	7.3
7	36.0	5.6	15.6
8	42.2	2.4	5.7
9	52.0	8.4	16.2
10	57.0	14.6	25.6
11	66.0	17.5	26.5
12	69.0	12.6	18.3
13	73.0	13.0	17.8
14	147.0	15.7	10.7
Mean	48.9	8.2	17.4
Std. Dev.	34.0	5.4	7.0
Sig. Diff. ("t" test):	No; $P > 0.10$	Yes; $P < 0.01$	Yes; $P < 0.01$

Patients with myxedema

Cerebrospinal fluid protein determinations on 14 myxedema patients without evidence of primary neurologic disease are also given in Table 1. Total protein concentration ranged from 20.0 to 147.0 mg. per 100 ml. In only 6 cases was an elevated value found. The mean of 48.9 mg. per 100 ml. was not significantly different from the control mean at the 10 per cent level employing the "t" test. On the other hand, gamma globulin values greater than 2 standard deviations from the control mean were found in 10 subjects; the mean of 8.2 mg. per 100 ml. was significantly different at the 1 per cent level. In 10 cases there were abnormally high ratios of gamma globulin/total protein; again the mean (17.4 per cent) was significantly different from the control mean at the 1 per cent level. No consistent relationship was found between the gamma globulin concentration or ratio and the severity of the clinical state, the etiology of the myxedema, or the absolute level of total cerebrospinal fluid protein.

Serum protein partition. Various alterations of serum protein components—decreased albumin, increased beta globulin, increased gamma globulin—have been described in cases of myxedema (3, 7–10). Particularly marked elevation of gamma globulin may be seen in Hashimoto's struma (10–12).

In the present studies, electrophoretic separation of the serum proteins was performed in all myxedema subjects. Using this method, 7 patients had a normal proportion of serum gamma globulin (7–17 per cent); the remaining 7 had a moderate (19.8 per cent) to marked (44.2 per cent) increase in gamma globulin. This latter group consisted of 3 patients with idiopathic atrophy of the thyroid, 2 with Hashimoto's struma, and 2 siblings with goitrous cretinism. In Table 2 the serum gamma globulin percentage determined by electrophoresis is compared to spinal fluid gamma globulin percentage determined by tyrosine equivalence, for each patient with myxedema. There was no significant linear correlation between the percentage of gamma globulin in the serum and in the cerebrospinal fluid. However, the applicability of this observation is questionable, since the two analytic methods are not directly comparable. In particular, the discrepancy between the affinities of albumin and the globulins for bromphenol blue dye would lead to varying degrees of underestimation of the serum gamma globulin percentage by the electrophoretic method (13). In contrast, the tyrosine equivalence method would yield quantitative recovery of both total protein and gamma globulin from spinal fluid within the range of values found in the present study (4).

Effect of treatment. Analyses of cerebrospinal fluid and serum were repeated in Patient M.S. after thirty-two weeks, and in Patient E.B. after twenty-one weeks of thyroid replacement therapy (Table 3). Despite the

TABLE 2. COMPARISON OF CSF AND SERUM GAMMA GLOBULIN IN 14 MYXEDEMA PATIENTS

Subject No.	γ-Globulin/Total protein (%)	
	CSF	Serum
1	17.5	31.0
2	29.0	23.7
3	23.1	19.8
4	11.5	12.2
5	18.6	13.6
6	7.3	13.2
7	15.6	12.2
8	5.7	11.4
9	16.2	44.2
10	25.6	20.0
11	26.5	16.9
12	18.3	23.2
13	17.8	21.3
14	10.7	15.6

Correlation: No; $r = 0.30$. $P > 0.10$.

fact that neither patient was rendered completely euthyroid, the concentrations of total protein and gamma globulin in the cerebrospinal fluid had fallen strikingly in both. The gamma globulin/total protein ratio was normal prior to therapy in Patient E.B., and markedly elevated in Patient M.S. However, in both the ratio decreased after therapy. The significance of this change cannot be assessed from the few data available. Fortuitously, the serum gamma globulin percentage increased slightly following thyroid therapy in both cases, demonstrating again the lack of correlation between alterations in serum and spinal fluid gamma globulin.

TABLE 3. EFFECT OF THYROID REPLACEMENT THERAPY ON CSF PROTEINS

	Total protein (mg./100 ml.)	γ-Globulin (mg./100 ml.)	γ-Glob./Tot. prot. (%)
M.S., age 55, white female (Case 11)			
July 18, 1958	66.0	17.5	26.5
Feb. 27, 1959	45.0	9.4	20.9
E.B., age 48, white female (Case 14)			
Sept. 3, 1958	147.0	15.7	10.7
Jan. 26, 1959	84.0	7.2	8.6

DISCUSSION

In previous reports on the elevated level of cerebrospinal total protein in myxedema the data have been based on an absolute increase in both albumin and globulin concentrations, as determined by simple qualitative testing (2) and by electrophoretic partition (3). It was concluded that altered capillary permeability in myxedema accounted for the high protein concentration in the cerebrospinal fluid (1), and that increased capillary permeability at the blood-cerebrospinal fluid barrier accounted for the increase in all spinal fluid protein components in direct proportion to their concentration in serum (3).

In the present studies on cerebrospinal fluid, gamma globulin was increased out of proportion to total protein in 10 of 14 cases of myxedema. An absolute elevation of the gamma globulin level was found in 4 of 8 myxedema patients with a normal level of total protein. If increased permeability of the blood-cerebrospinal fluid barrier to serum protein components were the cause, one might predict—on the basis of protein molecular size—a proportionally greater increase in albumin, hence in total protein, than in globulin (14).

There are several alternate explanations : 1) Serum hypergammaglobulinemia may produce parallel changes in cerebrospinal fluid (15, 16); such a correlation could not be established in these experiments. 2) An alteration in cerebrospinal fluid drainage may result in acceleration of albumin removal, a retardation of globulin removal, or both (14), leading to an increase in the gamma globulin ratio, and possibly in the absolute gamma globulin concentration. 3) The gamma globulin may originate directly from nervous tissue or supporting structures. This phenomenon is believed to occur in other disease states, *e.g.*, neurosyphilis (15, 16) and multiple sclerosis (16, 17).

Acknowledgment

The authors sincerely appreciate the advice and encouragement received during this study from Dr. Laurence H. Kyle of the Department of Medicine and Dr. Walter C. Hess of the Department of Biological Chemistry, Georgetown University Medical Center.

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TWO CASES OF GRAVES' DISEASE WITH SIGNS OF A PYRAMIDAL LESION. IMPROVEMENT IN NEUROLOGIC SIGNS DURING TREATMENT WITH ANTITHYROID DRUGS

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ABSTRACT

Two cases of Graves' disease with concomitant symptoms of pyramidal irritation are reported. Treatment with antithyroid drugs alleviated both the endocrine and the neurologic signs and symptoms. This is the main argument in favor of the neurologic disorder being of thyrotoxic origin.

THIS report concerns 2 patients in whom signs of a pyramidal lesion appeared in the course of Graves' disease. The neurologic signs and symptoms disappeared almost entirely in association with improvement in the hyperthyroidism during treatment with antithyroid drugs.

CASE REPORTS

Case 1

(*No. 16,545*). A 50-year-old woman was seen in June 1957 because of palpitation, nervousness, fatigue and loss of weight. She had difficulty in walking owing to weakness of the lower limbs. This condition had developed over a period of about four weeks. The patient was in the menopause and had had climacteric symptoms (hot flushes and tingling of the hands) for a year and a half. The mother and 2 cousins had goiter.

Physical examination revealed typical Graves' disease, with exophthalmos, bilateral Graefe's sign, diffuse goiter, tachycardia (152 beats per minute, regular), cardiovascular instability, palpitation, and tremor of the fingers. Mentation and speech were normal. The only abnormality in the cranial nerves was an increased masseter reflex. Pupillary reflexes were normal. There was slight hypotonus in the upper limbs. Muscle volume was preserved, and strength was normal in all sectors. Tendon reflexes were exaggerated on both sides, and on the left there was an increase in the reflexogenic area for the supinator reflex. Hoffmann's sign could be elicited bilaterally. In the lower limbs there was moderate wasting of both quadriceps. Plummer's and Lahey's signs were present. Passive movement revealed hypotonus of the psoas, iliacus and quadriceps muscles;

Received September 8, 1959.

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strength was decreased in the quadriceps and in the adductors and abductors of the thighs, but was preserved in the anterior and posterior muscles of both legs. Tendon reflexes were brisk. There was an increase in the reflexogenic area for the knee jerks, and brief clonus of both patellae. The ankle jerks and medial plantar reflexes were brisk and there was prolonged bilateral ankle clonus. Babinski's sign was present bilaterally. Walking was pareto-spasmodic. There was no ataxia. Sensory function was unimpaired, and the ocular fundi were normal.

Laboratory findings. On June 12, 1957, the basal metabolic rate (BMR) was +67 per cent, and the blood cholesterol level was 136 mg. per 100 ml. The erythyrocyte count was 3,200,000 per cu. mm., hemoglobin 65 per cent, color index 1.01, and the leukocyte count 6,900 per cu. mm. (neutrophils 60 per cent, monocytes 5 per cent, lymphocytes 35 per cent). The urine and cerebrospinal fluid were normal. A radiograph of the spinal column revealed osteoporosis.

Conclusion: No signs of a peripheral nerve lesion.

Clinical course. On June 23, 1957, her weight was 49 Kg. The pulse was 120, and regular. Treatment was begun with 40 mg. of methimazole (Tapazole) per day, but was discontinued after only two days because leukopenia developed. Therapy was started again on July 16, 1957, with 300 mg. of propylthiouracil daily. The BMR was +20 per cent and the blood cholesterol level 230 mg. per 100 ml.

In successive examinations from August 26, 1957 to October 11, 1957 the neurologic condition remained almost unchanged. In the upper limbs the tendon reflexes were brisk, especially on the right. On August 26, 1957, although there was hyperreflexia in the lower limbs, it was less marked than at the first examination and was predominantly on the right side, with an incomplete clonus of the patella. Although the weakness had improved slightly, Plummer's and Lahey's signs were still present, and walking was slightly pareto-spasmodic. There was slight hypotonus. The left knee jerk was brisker than the right, whereas the right ankle jerk was brisker than the left. At the time of the last two examinations ankle clonus had disappeared. The plantar flexor reflex was present on both sides.

In short, there had been a slight, bilateral pyramidal syndrome with moderate paresis of both lower limbs, and there had been partial but rapid improvement.

On October 22, 1957 a subtotal thyroidectomy was performed. This was followed by four days of high temperature and rapid pulse. The condition was interpreted as a severe thyrotoxic crisis and treated with refrigeration, sodium iodide and oxygen. Recovery was slow but complete.

By June 1958, her weight was 66.5 Kg., the BMR +6 per cent, and the blood cholesterol level 136 mg. per 100 ml. The upper limbs showed slight muscular hypotonus, but normal strength. Tendon reflexes were brisk, especially on the right side, but without any increase of the reflexogenic area. The lower limbs showed slight wasting of the vastus muscles and hypotonus of the adductors on both sides. The ankle jerks were brisk. There was no clonus of the foot or the patella. The Babinski sign had disappeared on both sides, and strength was normal.

Case 2

(*No. 16,819*). A 60-year-old man was admitted to the Endocrinological Institute in May 1957. A year before he had enjoyed normal health, weighed approximately 82 Kg., and did his farm work without effort. Without previous symptoms he contracted what he termed influenza, with chills, after which he felt depressed and unfit for work, and had no appetite. Two months later he began to experience difficulty in swallowing solid foods, especially bread and meat. Dysphagia increased steadily, until by August 1956

he could swallow only liquids. He also was irritable and restless, had a tremor of the hands, and noted exophthalmos, palpitation, and dyspnea on exertion. He consulted a physician who sent him to the hospital in November 1956. His weight was 58 Kg. He was in the hospital for twenty-five days, and was treated with iodine. By the time of discharge, nervousness and tremor had improved. The dysphagia became less severe, and he was able to eat soft foods, but not bread or meat. The asthenia did not improve. He continued to take iodine and tried to return to his usual work, but after fifteen days he could work no longer and again had dysphagia, tremor, nervousness, asthenia, slight diarrhea, dyspnea on effort and orthopnea. He could scarcely walk because of the asthenia and weakness. He was admitted to the hospital weighing 44 Kg. At no time did he sweat profusely, although he complained of the heat. He did not notice any swelling of the neck. There was edema of the legs, and impotence of one year's duration.

Physical examination on May 10, 1957 revealed an emaciated patient (47.4 Kg.) with an anxious appearance, dyspnea, hot hands and a rapid tremor of head and limbs. There was a bright staring gaze, normal pupils, horizontal nystagmus on extreme lateral vision, and bilateral exophthalmos. Dalrymple's and Graefe's signs were present. There was a small, firm painless diffuse goiter, with an intense bruit and thrill.

The pulse was 110, with complete tachyarrhythmia. The blood pressure was 115/60 mm. Hg. The apex beat of the heart was in the sixth intercostal space, lateral to the mid-clavicular line, and there was an apical systolic murmur. Mental function and cranial nerves were normal. The muscles showed generalized wasting and there was generalized loss of strength. There were no spontaneous or provoked fascicular contractions, and no muscular pain. Idiomuscular reflexes were normal. The tendon reflexes were brisk, especially on the left side, and Plummer's sign was present. There was incomplete patellar clonus on the left, and a Babinski response bilaterally. Barré's sign could not be elicited. Walking was difficult, owing to weakness. There were no changes of sensory function and no ataxia.

Laboratory findings. May 15, 1957, the BMR was +56 per cent, blood cholesterol level 132 mg. per 100 ml., erythrocyte count 3,260,000 per cu. mm., hemoglobin 65 per cent, color index 1.00, leukocyte count 7,700 per cu. mm. (neutrophils 70 per cent, monocytes 4 per cent, lymphocytes, 26 per cent), serum calcium level 9.5 mg. per 100 ml., serum sodium 299 mg. per 100 ml., and serum phosphorus 5.29 mg. per 100 ml. The electrocardiogram indicated auricular fibrillation. A radiograph of the chest showed an enlarged left auricle and ventricle. A radiograph of the esophagus showed a prolonged pharyngeal deglutition time; a large part of the ingested opaque substance remained in the folds of the mucosa and in the pyriform fossae, owing to hypoperistalsis. Parenteral injection of Prostigmine did not modify this condition.

Otorhinolaryngologic examination showed normal movements of the soft palate, tongue and larynx, and paresis of both the constrictor pharyngis inferior and medius; the pyriform fossae were filled with saliva. The pharyngeal mucosa was pale, but there were no proliferative or destructive lesions. Sensory function was preserved. On May 20, 1957, x-ray examination showed a normal stomach and duodenum; the gastric juice was normal. The right brachio-radialis and quadriceps were studied by electromyography. At the insertions of both muscles the reaction was normal, and no denervation potential was observed. During effort, there was a moderate but not abnormal increase in the number of polyphasic motor units.

Clinical course. The hyperthyroidism improved with three months' rest, and the weight increased by 7 Kg.

On August 31, 1957, propylthiouracil was prescribed in a dosage of 300 mg. daily. A few days after commencing this treatment, dysphagia improved and the patient was

able to swallow solid food. The weight increased by 3 Kg. Complete cardiac arrhythmia persisted.

On October 8, 1957, his weight was 62.2 Kg. The pulse was irregular (80 beats per minute), and he had a tremor of the fingers. Plummer's sign was still present. Tendon reflexes in the upper limbs were normal. The muscular wasting in the lower limbs had disappeared. There was weakness of the muscles that flex the leg upon the thigh, especially on the left side, but the strength of the adductors and abductors was preserved. Tendon reflexes were brisk and there was no clonus. Babinski's sign was present bilaterally. The BMR was +7 per cent, and the blood cholesterol level was 185 mg. per 100 ml. The cerebrospinal fluid was normal. No changes were demonstrated in the pharynx by x-ray examination. The electromyogram was unchanged.

On December 18, 1957, there was no dysphagia. Complete cardiac arrhythmia persisted, with a pulse of 80. The weight was 67.5 Kg. Plummer's sign had disappeared, as had all the neurologic signs except a doubtful Babinski response on the left side. The BMR was −5 per cent, and the blood cholesterol level was 260 mg. per 100 ml. X-ray examination showed that the pharynx was unchanged. The electromyographic findings were within normal limits. The patient was discharged taking 300 mg. of propylthiouracil daily.

On April 24, 1957, the patient was examined again. He had continued taking propylthiouracil irregularly, but had given it up completely during the preceding weeks. This led to the reappearance of the hyperthyroidism (*e.g.*, tachycardia, nervousness, Plummer's sign), but not the dysphagia. In the lower limbs the tendon reflexes were brisk on both sides, and there was a Babinski response bilaterally. The pulse was 88 and completely arrhythmic. The goiter was unchanged. The BMR was +51 per cent, and the blood cholesterol level was 182 mg. per 100 ml.

DISCUSSION

The first case was an example of Graves' disease plus a syndrome of pyramidal irritation consisting of a bilateral Babinski response, bilateral clonus of the ankle and patella, a brisk masseter reflex, and increased tendon reflexes in all four limbs.

On the other hand, signs of pyramidal paresis were absent in the upper limbs and doubtful in the lower, for although there was weakness of the lower limbs and difficulty in walking, these could both be explained by thyrotoxic myopathy apart from any affection of the pyramidal system. Moreover, Barré's sign, a very sensitive test of pyramidal paresis, was not observed.

These neurologic signs and symptoms improved considerably when the hyperthyroidism was corrected with antithyroid treatment. When the patient became euthyroid, Plummer's sign disappeared and the gait became normal, *i.e.*, the thyrotoxic myopathy was also cured.

The second case was also an example of Graves' disease, in the course of which generalized wasting of muscles of the four limbs, dysphagia and signs of pyramidal irritation supervened. The wasting of the muscles and the resulting weakness have been described previously but they are not common in this disease. Electromyography revealed an increase in the number of

polyphasic motor units, suggesting the possibility of thyrotoxic dystrophy of the muscles. This was confirmed by the disappearance of these abnormalities as the hyperthyroidism improved.

The second striking feature was dysphagia, which was shown fluoroscopically to be pharyngeal. What was the cause of this dysphagia? Examination revealed no ulcerative or proliferative lesions of the pharynx and, although the sensory function of this organ was intact, there was paresis of the constrictor pharyngis medium and the constrictor pharyngis interior. Since Graves' disease is sometimes associated with Erb-Goldflam's myasthenia, intramuscular and intravenous injections of Prostigmine and Mestinon were tried, but there was neither subjective nor radiologic improvement in the dysphagia. The dysphagia improved when the hyperthyroidism was treated, first with iodine and later with propylthiouracil. However, although dysphagia improved with treatment, no radiologic change was observed during deglutition. This discrepancy suggests the possibility that the improvement in the dysphagia during antithyroid therapy may have been a mere coincidence. The patient had probably been suffering from a myogenic dysphagia similar to those described by Madsen (1), together with generalized muscular dystrophia of thyrotoxic origin.

This patient presented a clear picture of pyramidal irritation which disappeared when thyroid function became normal. When treatment with propylthiouracil was discontinued, the signs of hyperthyroidism and the Babinski response reappeared.

These 2 cases of a pyramidal syndrome accompanying Graves' disease are of interest, because of their rarity. Cases have been published of hemiplegia, monoparesia or paraplegia in the course of Graves' disease. As Sattler (2) stated in 1909, in none of the cases discussed by him was there evidence that the neurologic disorders were cured by the antithyroid treatment—at that time almost exclusively thyroidectomy. On the other hand, in the 2 cases presented in this paper, the parallel improvement in the hyperthyroidism and the neurologic disorder indicate that the pyramidal symptoms were due to the hyperthyroidism.

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DEFECTIVE IODINATION OF TYROSINE A CAUSE OF NODULAR GOITER?*

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ABSTRACT

Twenty patients with colloid nodular goiter, 7 patients with Hashimoto's struma, and 15 patients without thyroid disease (all non-deaf) were tested with radioactive iodine and potassium thiocyanate for a thyroidal defect in iodination of tyrosine. In the cases of colloid nodular goiter with a family history of goiter there was a higher incidence and more severe degree of defective iodination of tyrosine than in the cases of colloid nodular goiter without a family history of goiter. Defective iodination of tyrosine was demonstrated in 1 sibling with proved colloid nodular goiter and in 1 with Hashimoto's struma. Iodination of tyrosine was impaired in 1 patient with her fourth recurrence of colloid nodular goiter. These data suggest that defective iodination of tyrosine is not uncommon in euthyroid non-deaf subjects with colloid nodular goiter, and may occur on a familial basis.

A TRACER dose of radioactive iodine (I^{131}) is normally incorporated into the tyrosine molecule shortly after entering the thyroid gland (1). If an oral dose of potassium thiocyanate (KSCN) is given to a person with a normal thyroid gland two hours after the administration of a tracer dose of I^{131}, the resultant decrease in concentration differential for ionic iodide across the follicular cell membrane does not result in an exodus of I^{131} from the thyroid gland because the I^{131} already incorporated into the tyrosine molecule does not leave the follicular cell. If the process of iodination of tyrosine is impaired, however, nonorganified I^{131} leaves the thyroid gland after the administration of KSCN. Defects in the organification of iodine have been noted during administration of thiouracil (2), and in goitrous cretins (3), congenitally nerve-deaf siblings in whom goiter later developed (4), and patients with Hashimoto's struma (5).

Received June 25, 1959.

* This work was aided by grants from the United States Public Health Service (M-2399), the Atomic Energy Commission (Project #15), and the Michigan Memorial Phoenix Project (#156).

We wish to present evidence that defective organification of iodine is not an uncommon finding in euthyroid non-deaf subjects with colloid nodular goiter, and that the defect may occur on a familial basis.

CLINICAL MATERIAL AND METHODS

Patients

Twenty-seven patients with nontoxic goiter and a control group of 15 subjects without thyroid disease were tested for a defect in organification of I^{131}. The data on these 42 patients are listed in Table 1. In the control group, 7 subjects were paid adult normal volunteers (chiefly medical students) and 8 were euthyroid nongoitrous patients. No person with a family history of thyroid disease was included in the control group. With the exception of the normal volunteers, all subjects included in Table 1 under any category were chosen from routine clinical material seen in the Clinical Radioisotope Unit, University Hospital, during the period from December 1958 through February 1959. All goitrous patients (other than those with clinical Hashimoto's struma) had nontoxic colloid nodular goiter, as judged by the medical history, physical examination, and by one or more of the conventional laboratory tests of thyroid function. Seven patients included in Table 1 had Hashimoto's struma; they were all females between 20 and 46 years of age, and had firm diffusely enlarged goiters that had not developed during puberty.

No patient was hypothyroid or deaf. When selecting goitrous patients, a particular effort was made to include those with a family history of the disease, *i. e.*, one or more of the parents, siblings, or the offspring of the propositus had a history of goiter. No two subjects included in Table 1, however, were relatives. Nine of 20 patients with colloid nodular goiter had a family history of goiter. The 7 subjects with Hashimoto's struma were tested to demonstrate our ability to detect a defect in organification of iodine in goitrous patients known to have a high incidence of this defect (5).

The clinical diagnosis of colloid nodular goiter was substantiated at the time of subtotal thyroidectomy in 12 of the 20 cases presented in Table 1; in the remaining 8 cases thyroidectomy was not performed. In the group with colloid nodular goiter, the 12 thyroidectomized patients comprised 7 of the 9 patients with a family history of goiter and 5 of the 11 patients without a family history of goiter. In 3 of the 7 cases of Hashimoto's struma, the clinical diagnosis was confirmed at the time of thyroidectomy. Among the 4 patients with Hashimoto's struma who were not treated surgically, 3 had circulating antithyroid antibodies as determined by the tanned red blood cell hemagglutination technique (6); the fourth patient had a defect in organification of iodine and the goiter disappeared upon administration of desiccated thyroid.

In addition to the clinical material presented in Table 1, four generations of one family with a strong history of goiter in three generations were studied. Pertinent details are presented in Figure 1. Seven members of two generations of this family were tested for a defect in the organification of iodine. One of the family members is included in the material presented in Table 1. This woman had a colloid nodular goiter, proved at the time of operation. One of her sisters had Hashimoto's struma, also proved at the time of operation. All members of this family were euthyroid. None was deaf.

One 72-year-old clinically euthyroid woman (not included in Table 1 or Figure 1) with a fourth recurrence of colloid adenomatous goiter after four surgical thyroidectomies was tested for defective organification of iodine. The level of serum protein-bound iodine (PBI) was 3.1 μg. per 100 ml. The diagnosis was proved by a fifth surgical thy-

roidectomy, performed because of severe compression symptoms. The results of the thiocyanate tests are presented in Figure 2.

Method of testing for a defect in thyroidal organification of iodine

One gram of potassium thiocyanate (KSCN) was given orally to the fasting patient two hours after an oral dose of from 10 to 30 μc. of I^{131}. Thyroidal I^{131} uptake measurements were made by the method of Brucer (7) using a thin lead shield over a scintillation detector to screen out low-energy radiation and a heavy lead shield over the neck to permit counting of body radioactivity minus radioactivity from the thyroid area. Uptakes at half an hour and one hour following the administration of KSCN were expressed as a positive or negative percentage change from the two-hour percentage uptake of the dose of I^{131}. Defective organification of iodine was judged to be present if the half-hour and one-hour post-KSCN values were each less than the two-hour value, and if either the half-hour or the one-hour uptake showed a drop greater than 10 per cent from the two-hour level. To insure statistical validity of radioactivity counting, no patient was included in this study whose thyroidal I^{131} uptake failed to reach 4 per cent within two hours after administration of I^{131}.

RESULTS

Incidence of defective thyroidal organification of iodine in colloid nodular goiter

The results of testing in the 4 major groups studied are summarized in Table 1.

There was a significant difference in the frequency of defective organification of iodine betwen the controls and the goitrous patients with a family history of goiter ($P < 0.005$). The frequency was not significantly different between the controls and the goitrous patients with no family history of goiter. The difference in frequency of defective organification of iodine between controls and the patients with Hashimoto's struma was significant ($P < 0.005$). Similarly, the differences in the mean percentage change in thyroidal I^{131} uptake at half an hour and at one hour after administration of KSCN were significant, comparing: a) normal subjects and goitrous patients with a family history of goiter (half hour, $P < 0.001$; one hour, $P < .001$), b) goitrous patients with a family history of goiter and goitrous patients without a family history of goiter (half hour, $0.05 < P < 0.1$; one hour, $0.025 < P < 0.05$), and c) normal subjects and patients with Hashimoto's struma (half hour and one hour, $P < 0.001$).

No correlations were observed between the size of the goiter or the rate of I^{131} uptake and abnormal results of the test.

Occurrence of defective thyroidal organification of iodine in members of one family

Figure 1 represents a partial study of four generations of one family with a high incidence of goiter. The propositus (A) had a defect in organifica-

TABLE 1. PERCENTAGE CHANGE FROM TWO-HOUR THYROIDAL I^{131} UPTAKE VALUE AFTER ADMINISTRATION OF 1 GM. OF KSCN ORALLY

Subjects	Time after administration of KSCN		Results of KSCN test
	½ hour	1 hour	
CONTROL GROUP			
Euthyroid patients			
B	+33	+33	−
M	+53	+55	−
L	+ 4	+11	−
B	+31	− 6	−
S	−12	+ 2	−
M	− 1	− 7	−
M	+27	−11	−
S	+20	+10	−
	Mean +19.4	+10.9	
Normal volunteers			
M	+ 6	+ 6	−
B	+20	+ 4	−
S	+56	+77	−
T	+13	+ 5	−
G	+31	− 8	−
R	+10	+29	−
K	+20	− 6	−
	Mean +22.3	+15.3	
	Mean for all controls: +20.7 (±18.7)*	+12.9 (±25.3)*	None positive
GOITROUS GROUP			
Colloid adenomatous goiter; family history of goiter			
A	− 6	−12	+
G	−10	−26	+
J	− 7	−36	+
U	−11	− 9	+
S	−56	−75	+
Sn	+12	+11	−
T	+ 4	+25	−
Y	+ 6	−1	−
St	−20	−22	+
	Mean −10	−17	6 positive

TABLE 1. (*Continued*)

Subjects	Time after administration of KSCN		Results of KSCN test
	½ hour	1 hour	
Colloid adenomatous goiter; no family history of goiter			
W	−25	− 8	+
B	− 1	+ 4	−
Br	− 5	+24	−
H	+24	+24	−
O	+16	+17	−
Ho	−25	0	−
Z	+ 2	− 7	−
Ba	+ 6	− 1	−
He	+16	+15	−
K	+25	− 2	−
R	+67	+106	−
	Mean + 9	+15.6	1 positive
Hashimoto's struma			
C	−15	−25	+
S	−22	−33	+
G	−11	−24	+
B	−77	−69	+
T	−21	− 9	+
St	+14	+10	−
Se	−35	− 8	+
	−24	−23	6 positive

* Standard deviation.

tion of iodine; subsequently the diagnosis of adenomatous goiter was proved at operation. One goitrous sibling (H) had a defect in organification of iodine; at operation the diagnosis of Hashimoto's struma was confirmed. Two nongoitrous siblings and 1 nongoitrous offspring of one of these siblings (listed in Figure 1 as showing a positive result with the KSCN test) did not react positively to the test by the criteria described. However, they responded to KSCN in a manner different from that of the controls, and so are listed here for interest. Their post-KSCN thyroidal I^{131} uptake values were depressed 10 per cent or more on two successive determinations whereas the 11 control subjects who had comparable determinations did not exhibit this phenomenon.

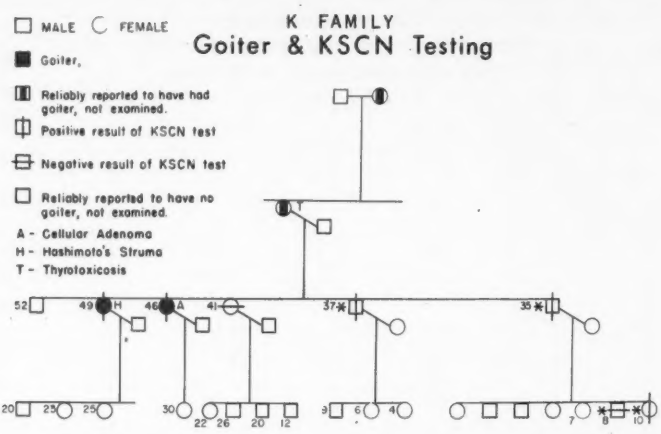

FIG. 1. Family of four generations. Three generations are known to have goiters. In some of the members of 2 generations the effect of KSCN on the thyroidal uptake of I^{131} was determined in order to test for a defect in organification of iodine. The numbers indicate the patients' ages. The criteria for interpreting the results of the KSCN tests are described in the text. Asterisks indicate patients in whom *any 2 consecutive* I^{131} uptakes after KSCN were $\leq 90\%$ of the uptake before KSCN. (In 11 controls in whom data were obtained for comparison, no 2 *consecutive* uptakes after KSCN were depressed to this degree.)

Figure 2 shows the rate and degree of discharge of I^{131} from the thyroid gland of the 72-year-old woman with a fourth recurrence of surgically proved colloid adenomatous goiter. This I^{131} discharge curve is contrasted with the mean I^{131} percentage uptake curve after KSCN in the 15 control subjects. It is evident that in the average control subject there was no de-

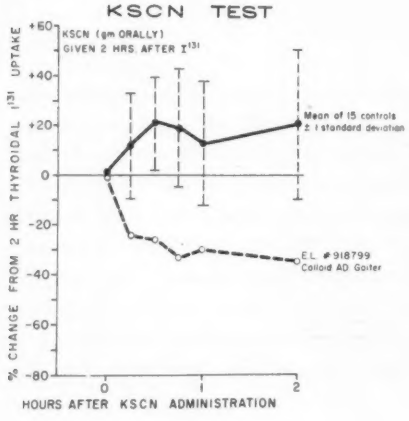

FIG. 2. Results of testing for a defect in organification of iodine in a patient with a fourth recurrence of colloid adenomatous goiter, compared to the results (mean values) obtained in 15 patients without thyroid disease.

crease in the count of radioactivity over the thyroid gland after administration of KSCN. In the patient with recurrent goiter there was a marked decrease in thyroidal I^{131} content after administration of KSCN.

DISCUSSION

Our data demonstrating that there is an increased incidence of defective thyroidal organification of iodine in patients with colloid adrenomatous goiter having a strong family history of goiter raises the possibility that a familial partial defect in organification of iodine may give rise to colloid nodular goiter in humans. The finding that, in a group of patients with colloid nodular goiter, this defect occurs more commonly in those with a family history of goiter than in those without this history, makes it unlikely that the defect exists only in the nodule or adenoma.

If a familial or hereditary defect in thyroidal iodination of tyrosine is a cause of colloid nodular goiter, the mechanism of goiter development could be visualized as follows. A partial familial or hereditary defect in organification of iodine would tend to result in the formation of less than normal amounts of thyroid hormone. A subnormal supply of thyroid hormone acting on the pituitary gland or hypothalamus might increase the activity of the thyroid-stimulating hormone (TSH). Thyroid morphology would be affected by smaller variations in iodine intake (8) in the presence of a partial defect in hormonal synthesis. Colloid nodules would then develop over a period of time (8). Follis (9) produced colloid nodular goiters in rats by impairing the thyroidal iodination of tyrosine through the administration of propylthiouracil.

The fact that a familial partial defect in organification of iodine can exist without clinical evidence of hypothyroidism or perhaps without goiter raises the possibility that euthyroid nongoitrous parents of cretins with a demonstrated defective organification of iodine may also have defective thyroidal iodination of tyrosine and may transmit this defect to their offspring, some of whom may thus be cretins.

A defect has been demonstrated in the organification of iodine in a woman with a fourth postoperative recurrence of proved colloid adenomatous goiter and with a low-normal to mildly hypothyroid level of serum protein-bound iodine. This raises the possibility that in some of the 22 per cent of nontoxic goiters that recur after surgical thyroidectomy (10), defective organification of iodine may be the etiologic factor.

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EFFECT OF METHYLTHIOURACIL AND IODIDE ON THE IODINATED CONSTITUENTS OF THYROID TISSUE IN GRAVES' DISEASE

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ABSTRACT

The distribution of radioiodine amongst the various iodinated constituents of thyroid tissue was determined chromatographically in patients with Graves' disease who had been treated with methylthiouracil for twenty days and then with iodide alone for ten days before thyroidectomy. Specimens of normal tissue from euthyroid patients with adenoma of the thyroid were used as controls. In the tissue from patients with hyperthyroidism the proportion of inorganic radioiodide was higher (28.8 per cent) than normal (8.1 per cent) and the ratio of radiomonoiodotyrosine to radiodiiodotyrosine (M/D) was higher (2.2) than normal (0.68). The latter could be explained in most cases by a block in iodine organification leading to a deficiency of iodine for the formation of diiodotyrosine. In 2 hyperthyroid patients, however, there was no apparent block in the organification of iodine. This block in the conversion of monoiodotyrosine to diiodotyrosine in the hyperthyroid human subject might have been the result of iodide therapy, but it seems more probable that it was a long-lasting effect of methylthiouracil.

IT HAS recently been shown that propylthiouracil can inhibit the conversion of monoiodotryosine to diiodotyrosine in the thyroid of the normal rat (1, 2). This effect may last as long as four days after stopping propylthiouracil (2). Large doses of iodide can also inhibit the conversion of monoiodotyrosine to diiodotyrosine in the normal rat (3) but the duration of this effect seems to be brief (2, 3).

The present study shows the effect of a combination of an antithyroid compound (methylthiouracil) and iodide in inhibiting the conversion of monoiodotyrosine to diiodotyrosine in the thyroid gland of a patient with Graves' disease.

MATERIALS AND METHODS

The study included 21 patients with Graves' disease treated with methylthiouracil (5 to 6 Gm. in about twenty days) followed by Lugol's solution alone (120 drops in

Received October 22, 1959.

about ten days), and 8 euthyroid patients with adenoma of the thyroid. Diagnosis was made on the basis of the clinical picture, basal metabolic rate (BMR), thyroidal uptake of radioiodine, and the plasma level of protein-bound iodine (PBI). Seventy-two hours before thyroidectomy each patient was given 300 microcuries of radioiodine (I^{131}) by mouth. Thyroidal uptakes of radioiodine were again determined twenty-four hours later. Treatment with Lugol's solution was continued until the time of surgery. Following thyroidectomy fresh samples of the glands were homogenized and subjected to hydrolysis with 4 per cent pancreatin for twenty-four hours at 37° C. in Krebs-Ringer phosphate buffer at pH 7.4. The hydrolysate was concentrated by evaporation in a vacuum at 40° C. An aliquot of this concentrate was placed directly on paper and chromatographed by an ascending solvent system of butanol and 2*N* acetic acid. The spots of radioactivity determined by radioautography were cut out and counted in a scintillation well counter. All data are expressed in terms of radioactivity, as no chemical iodine determinations were performed, and no corrections were made for the possibility of two radioactive iodine atoms being associated with some diiodotyrosine molecules as compared to a maximum of one on monoiodotyrosine. The iodinated constituents of the thyroid are expressed as a percentage of the total radioactivity on the chromatographic strips. The total does not add up to exactly 100 per cent, as the interspot areas were not discretely counted.

RESULTS

Specimens of normal tissue from the adenomatous thyroids of the 8 euthyroid patients were used as a control series. These histologically normal tissues contained 2.3–18.6 (mean, 8.1) per cent inorganic radioiodide, 18.5–37.9 (mean, 28.1) per cent radiomonoiodotyrosine and 34.0–51.7 (mean, 42.0) per cent radiodiiodotyrosine (Table 1). In comparison, thy-

TABLE 1. DISTRIBUTION OF I^{131} IN HISTOLOGICALLY NORMAL THYROID TISSUE*

Patient			Distribution of radioactivity (% of total butanol −2*N* acetic acid)**							
No.	Name	Age & Sex	Origin	I	MIT	DIT	M+D	Ratio M/D	T_3+T_4	F
1	N.H.	29 F	4.4	2.3	29.3	41.9	71.2	0.69	10.1	3.8
2	W.S.	30 F	14.5	5.8	18.5	46.2	64.7	0.40	3.4	4.2
3	K.K.	54 F	7.0	3.7	27.6	51.7	79.3	0.53	5.9	2.6
4	M.K.	33 F	9.1	2.5	30.8	43.9	74.7	0.70	3.2	3.2
5	M.T.	50 F	7.9	5.1	37.9	42.9	80.8	0.88	3.6	3.2
6	N.M.	45 M	11.9	9.8	22.8	37.4	60.2	0.61	6.5	6.7
7	M.Y.	46 F	12.7	17.2	20.5	34.0	54.5	0.61	5.5	2.2
8	I.T.	60 F	5.9	18.6	37.6	38.2	75.8	0.98	3.5	2.5
Mean				8.1	28.1	42.0	70.1	0.68	5.2	
Standard Deviation				6.3	2.1	5.2		0.12	2.1	

* Obtained from the adenomatous thyroids of euthyroid patients.

** Abbreviations: I = Iodide. MIT = Monoiodotyrosine. DIT = Diiodotyrosine. T_3 = Triiodothyronine. T_4 = Thyroxine. F = Unknown spots at the front.

TABLE 2. DISTRIBUTION OF I^{131} IN THE THYROID TISSUE FROM 21 PATIENTS WITH TREATED GRAVES' DISEASE*

Patient			Thyroidal I^{131} uptake (% dose)		BMR (%)		Distribution of radioactivity (% of total butanol −2*N* acetic acid‡							
No.	Name	Sex & age	BM†	BO†	BM†	BO†	Origin	I	MIT	DIT	M+D	Ratio M/D	T_3+T_4	F
1	Y.T.	F 25	52.5	1.5	+34	+37	7.8	15.8	24.1	25.6	50.2	1.0	10.9	10.2
2	K.K.	F 36	59.4	1.9	+37	+22	1.9	19.8	27.3	8.8	36.1	3.1	16.5	12.0
3	S.E.	F 32	80.6	2.8	+17	+25	3.5	30.9	35.0	12.8	47.8	2.7	14.0	2.0
4	M.A.	F 23	76.7	3.0	+17	+ 7	2.6	4.8	36.2	21.2	57.3	1.7	18.2	10.1
5	N.Y.	F 36	63.0	3.5	+33	+29	4.6	21.4	27.2	25.6	52.8	1.1	8.7	7.7
6	F.K.	M 53	70.3	3.7	+33	+29	8.9	23.0	22.5	13.9	36.4	1.6	14.2	8.9
7	Z.K.	F 30	96.4	4.1	+24	+24	2.6	13.5	36.0	29.9	65.9	1.2	7.3	7.7
8	H.G.	M 26	50.4	4.2	+23	+24	5.5	7.9	29.4	35.2	64.6	0.8	15.1	2.0
9	N.K.	M 32	76.4	4.9	+44	+18	12.4	24.3	37.0	12.7	49.7	2.9	6.4	3.1
10	M.T.	F 37	46.1	5.1	+32	+23	4.3	35.7	16.5	20.4	36.9	0.8	7.6	7.4
11	Y.H.	F 29	79.7	5.5	+39	+33	5.3	18.5	31.6	12.4	44.0	2.6	16.1	8.2
12	A.Y.	F 31	87.7	6.6	+23	+ 7	5.2	9.1	57.8	15.2	73.0	3.8	4.9	2.0
13	N.T.	F 50	68.5	7.5	+29	+24	7.8	19.8	28.2	21.4	49.6	1.3	9.4	5.2
14	S.K.	M 37	44.1	9.4	+22	+13	1.8	57.7	25.2	9.2	34.6	2.7	3.7	
15	G.H.	M 57	53.2	9.7	+23	+ 7	0.5	89.5	7.9	1.4	9.3	5.2	0.5	
16	M.K.	F 44	70.0	9.8	+45	+34	6.3	17.6	33.7	13.5	47.2	1.5	10.9	
17	S.S.	M 49	72.8	10.8	+27	+32	4.5	1.7	29.2	37.0	66.2	0.8	9.4	
18	W.I.	F 30	52.0	12.5	+57		0	65.2	16.2	3.3	22.5	2.6	4.3	
19	Y.M.	F 21	77.6	13.0	+43	+24	0	48.4	17.8	3.6	21.4	4.5	3.3	4.6
20	M.K.	F 23	69.5	15.8	+25	+18	13.2	20.0	41.2	17.3	58.5	2.4	4.9	
21	T.T.	M 23	63.5	17.8	+49	+11	1.0	60.6	26.0	8.2	34.2	3.2	1.8	
Mean					+32	+22		28.8	28.9	16.5	45.6	2.2	8.9	
Standard Deviation									10.5	9.9		1.25	5.17	

* Treated with methylthiouracil for 20 days, followed by iodide alone for 10 days.
† BM = Before medication. BO = Before operation.
‡ For key to abbreviations, see footnotes to Table 1.

roid tissue from patients with Graves' disease treated as described contained 1.7–89.5 (mean, 28.8) per cent inorganic radioiodide, 7.9–57.8 (mean, 28.9) per cent radiomonoiodotyrosine and 1.4–3.0 (mean, 16.5) percent radiodiiodotyrosine (Table 2). The thyroidal uptake of radioiodine in the patients with treated Graves' disease was low before surgery as they were receiving stable iodide. The ratio of radiomonoiodotyrosine to radiodiiodotyrosine (M/D) in normal thyroid tissue was 0.4–0.98 (mean, 0.68), whereas in the thyroid tissue of patients treated for Graves' disease the M/D was 0.8–5.2 (mean, 2.2). The mean total percentage of radiomonoiodotyrosine plus diiodotyrosine was 70.1 for the normal glands and 45.6 for the hyperthyroid glands. In the 4 patients with Graves' disease in whose glands the percentages of radioiodide were below or close to the mean of the normal (Nos. 4, 8, 12 and 17), the M/D was 1.7, 0.8, 3.8 and 0.8. In Patients 4 and 12 the M/D was abnormally high in spite of a normal percentage of radioiodide. The sum of radiotriiodothyronine (T_3) and radiothyroxine (T_4) was often higher (mean, 8.9 per cent) in the hyperthyroid patients than in the normal subjects (mean 5.2 per cent). In the solvent system used it is not possible to separate these compounds adequately.

In 6 patients, t-amyl alcohol saturated with 2*N* ammonium hydroxide (which separates T_4 and T_3) was used, but no consistent difference in T_3/T_4 ratios was noted. It has become clear recently that an unknown compound often runs with an Rf similar to that of T_3 in these solvent systems. This material has not been identified, and the T_3 and T_4 results are therefore not interpretable. Whether there is a real difference between T_4 and T_3 in the hyperthyroid and the normal glands remains to be resolved.

DISCUSSION

In most of the patients with Graves' disease who had been treated first with methylthiouracil and then with iodide the organification of radioiodide was partially inhibited. The percentage of total radioactivity present as radioiodide was higher than normal. Iodide can inhibit the organification of iodide in the thyroid (4, 5) but the duration of this effect is brief in the rat (4). The duration of inhibition must usually be limited in the normal human subject since myxedema develops so rarely in patients treated with iodide (6). The duration of the iodide effect on the organification of iodine in hyperthyroidism is unknown. A high intrathyroidal iodide content has been described as long as ten days after the start of iodide therapy (7) in hyperthyroid patients. In the present study iodide was given for ten days before surgery.

The effect of antithyroid compounds in reducing the thyroidal uptake of radioiodine in rats begins to wear off within hours after a single injection (8). The inhibition of the conversion of monoiodotyrosine to diiodotyrosine by propylthiouracil (a reaction more sensitive to propylthiouracil than is organification of iodine) sometimes lasts at least four days in the normal rat (2). In the hyperthyroid human subject the thyroidal uptake returns to levels above normal within a few days of stopping therapy. In the present study it is not possible to say whether the inhibition of iodine organification in most of the hyperthyroid cases was due to a residual effect of methylthiouracil therapy, a prolonged effect of continuing iodide therapy, or a combination of both.

The high ratio of radiomonoiodotyrosine to radiodiiodotyrosine (M/D) found in most of the cases could be explained by a relative iodine deficiency. It has been shown that iodine deficiency leads to the formation of more monoiodotyrosine than diiodotyrosine (9). A continuing block in organification could simulate such an iodine deficiency. It is clear, however, that in 2 patients (Nos. 4 and 12, Table 2) there was no evidence for any reduction in organification of iodine. The increase in M/D in these cases could be the result of the iodide treatment, as has been demonstrated by Galton and Pitt-Rivers (3) in the rat. This effect is short-lived in the rat (2). It is not clear how long it may last in the human subject,

but Braasch and associates (7) found no increase in M/D after seven to ten days of iodide therapy in patients with exophthalmic goiter. It would seem more probable, then, that these 2 patients demonstrated a long-lasting effect of methylthiouracil in inhibiting the conversion of monoiodotyrosine to diiodotyrosine in the hyperthyroid human subject—an effect recently ascribed to propylthiouracil in the normal rat (1, 2).

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BUTANOL-INSOLUBLE IODINATED COMPOUND IN THE PLASMA OF A GOITROUS CRETIN*

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ABSTRACT

The case study is presented of a goitrous cretin in whom the only circulating iodinated material was butanol-insoluble. Radiochromatographic studies indicated that this material was hydrolyzed during its excretion through the kidney but was not broken down while in the general circulation.

THE production, and release, into the peripheral circulation of an iodinated polypeptide or protein by the thyroid gland in one type of goitrous cretins is well documented (1, 2). This iodinated substance, which differs from thyroglobulin (1), has no metabolic activity and does not suppress the production and release of thyroid-stimulating hormone (TSH), with the result that such patients have hypothyroidism and goiter.

In the reported cases, the severity of the hypothyroidism has varied from mild (2) to severe (3) and the degree of hypothyroidism has seemed to be related to the amount of thyroxine (T_4) and triiodothyronine (T_3) present. T_4 and T_3 as well as monoidotyrosine (MIT) and diiodotyrosine (DIT) were identified in the plasma of these patients (1–3); however, not all 4 iodinated amino acids were identified in all cases, and in some instances the amounts of T_4 and T_3 were insufficient for unequivocal identification although they were thought to be present (1). In some of the cases in which urinary studies were carried out, the iodinated tyrosines (MIT, DIT) and the iodinated thyronines (T_4, T_3) were identified in the urine (1, 3). Enzyme hydrolysis of the circulating iodinated protein or polypeptide, which is butanol-insoluble, yielded iodinated tyrosines as well as the iodinated thyronines (1).

It has not been clearly determined whether the presence of iodinated

Received December 7, 1959.

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amino acids in the blood of such patients is due to breakdown of iodinated proteins in the periphery, or to secretion by the thyroid gland. For this reason we thought it of interest to report the study of a patient with severe cretinism and goiter in whom the entire circulating iodinated material was butanol-insoluble.

CASE REPORT

A 23-year-old woman (Fig. 1), born and raised in a small Mexican town ten miles from the Pacific Coast, was admitted to the Mexico City General Hospital because of a goiter. Goiter is not endemic in this area. The patient's mother had a small goiter, and she stated that neither her 4 older children, nor any other family member had a goiter. The patient was the product of an uneventful pregnancy and delivery. Her growth and development were slow; the first teeth appeared at 7 years, she started walking and talking at 10 years, but her vocabulary was always limited to three or four words. She was an idiot, her mental age being 15 months. Constipation was always a problem and she had never menstruated.

FIG. 1. A 23-year-old female goitrous cretin.

Her height was 1.07 meters (height-age, 5 years), and she weighed 20.5 kilograms. She had cretinous facies and a large irregular goiter. Breast development was that of early puberty, but she had no pubic or axillary hair. The abdomen was protruding and there was an umbilical hernia of moderate size.

A roentgenogram of the skull revealed lack of pneumatization of the mastoid, frontal and sphenoid bones. The sella turcica was normal. The bones showed generalized osteoporosis and a bone-age of three years (Greulich and Pyle (4)), with fragmentation of some of the centers of ossification (epiphyseal dysgenesis). A roentgenogram of the chest showed pancardiac enlargement.

The level of serum protein-bound iodine (PBI) was 3 μg. per 100 ml. The thyroidal

accumulation of I^{131} was rapid, being 72 per cent of the dose at two hours, 90 per cent at three hours and 58 per cent at twenty-four hours. Two grams of potassium thiocyanate, given two hours after the ingestion of I^{131}, did not cause loss of iodide from the thyroid gland. Twenty-four hours after I^{131} ingestion, 92 per cent of the plasma activity was precipitable with trichloracetic acid but only 10 per cent of the activity could be extracted with n-butanol (normally 75–80 per cent of the activity is extractable with n-butanol).

Plasma samples were obtained three hours and twenty-four hours following an oral dose of 1 millicurie of I^{131}. Whole plasma and the n-butanol extracts of plasma were chromatographed in the butanol-ethanol-ammonia, acetic acid-water, and butanol-tertiary amyl alcohol systems. The chromatogram of the whole plasma from a blood sample drawn three hours after administration of I^{131} showed activity at the origin and at iodide, whereas the chromatogram of the butanol extract of this plasma showed activity only at iodide. The chromatogram of the whole plasma twenty-four hours after ingestion of I^{131} showed activity at the origin and only a small amount of activity at iodide; that of the butanol extract showed activity only at iodide. The chromatograms of each plasma sample yielded identical findings when developed in the various solvent systems, and in no instance was activity demonstrated at the stable markers for MIT, DIT, T_4 or T_3 (Fig. 2).

The chromatogram of urine collected during the 24-hour period following ingestion of I^{131} showed butanol-insoluble, non-moving material as well as activity at the stable markers for MIT and DIT, and to a lesser extent at those for T_4 and T_3. The activity running with the stable markers for the iodinated tyrosines and thyronines was eluated and rechromatographed in other systems and in each instance ran with the stable markers for these amino acids.

Chromatograms of plasma subjected to trypsin hydrolysis showed that the butanol-insoluble material was broken down into MIT, DIT, T_4 and T_3. Electrophoretic analysis showed that the butanol-insoluble material migrated with albumin.

DISCUSSION

The severity of the cretinism in this case indicated that metabolically active thyroid hormones had not been present in any degree for most of the patient's life. The biochemical data showed that the thyroid gland was capable of trapping iodide and organifying it, but the iodinated material released into the peripheral circulation was a metabolically inert butanol-insoluble iodinated protein or polypeptide. This iodinated material was similar to that reported by others in such cases, in that it migrated with albumin during electrophoresis and yielded iodinated tyrosines and thyronines upon trypsin hydrolysis; it was broken down into these same iodinated amino acids during its excretion through the kidney.

The clinical picture and the results of the biochemical studies in our case differ only in degree from those reported in similar cases of goitrous cretins (1, 3). Our patient was more severely retarded than those reported to date and we were unable to identify either tyrosines or thyronines in the circulation, whereas in the previously reported cases various combinations of these iodinated amino acids were identified. In view of the large dose of

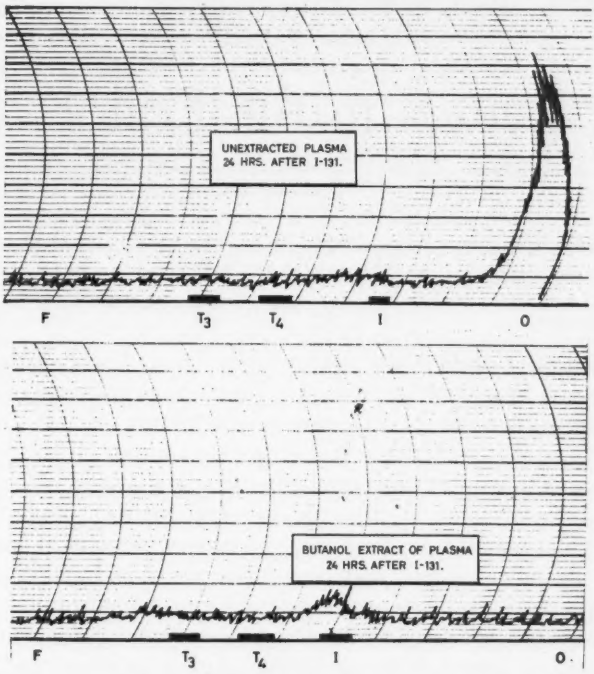

FIG. 2. Radiochromatograms (butonal-ethanol-ammonia system) of plasma from a goitrous cretin. Upper graph, whole (unextracted) plasma. Lower graph, n-butanol extract of plasma.

I^{131}, it is unlikely that we would have failed to find activities corresponding to the tyrosines or thyronines if these had been present in even minimal amounts.

The data in our case seem to indicate that hydrolysis of the butanol-insoluble protein did not occur until the material was being excreted. Moreover, the defect in the thyroid was so severe that no iodinated material other than the protein or polypeptide was produced, and this material was not hydrolyzed in the peripheral circulation.

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Letters to the Editor

SPECIES SPECIFICITY OF STEROID-INDUCED FEVER

To the Editor:

Previous studies from these laboratories have demonstrated that a number of steroid metabolites of the 5β-H type (pregnane and etiocholane) derived from adrenocortical and gonadal hormones provoke fever when administered intramuscularly or intravenously to man (1–4). These pyrogenic hormonal transformation products include C19 and C21 steroids of both the 11-desoxy and 11-oxygenated series such as: 3α-hydroxyetiocholan-17-one; pregnane-3α,20α-diol; 3α-hydroxypregnan-20-one; 3α,11β-dihydroxyetiocholan-17-one; and 3α-hydroxypregnan-11,20-dione. These studies established the existence of a new form of biologic activity for a class of compounds previously considered physiologically inert and defined a new category of potent fever-producing substances of endogenous origin in man.

Further studies of the biologic properties of these compounds have led to the demonstration of an apparent high degree of species specificity of steroid-induced fever. 3α-Hydroxypregnan-11,20-dione, a metabolite of endogenous hormones (5), is intensely pyrogenic when administered by intramuscular or intravenous injection to man in doses of approximately 0.2 to 1.0 mg. per Kg. of body weight (3, 4). This steroid, dissolved in small amounts of sesame oil-benzyl alcohol vehicle (2), was given by intramuscular injection in doses of 1.0 and 5.0 mg. per Kg. to between 8 and 12 animals of each of the following species: rat, mouse, guinea pig, dog, cat, rabbit and monkey (M. Rhesus). In addition, 2 animals of each of the 4 latter species were given intravenous injections of the steroid (in small amounts of ethyl alcohol) in doses of 1.0 mg. per Kg. In order to demonstrate that the laboratory environment and conditions of mild physical restraint and animal handling used in these experiments did not inhibit the production of fever, these animals were later given intravenous injections of bacterial pyrogen (Piromen). Temperatures were measured rectally by means of indwelling thermistor probes and were recorded continuously during appropriate control periods and for eighteen to twenty-four hours after injections.

Injection of the steroid pyrogen, 3α-hydroxypregnan-11,20-dione, did not provoke significant elevation of temperature in any of the animal species studied despite the fact that it was administered in doses comparable to or greatly exceeding those demonstrated to provoke intense fever consistently in man. Under the same experimental conditions, administration of bacterial pyrogen did provoke fever in susceptible species (dog, cat, rabbit, monkey), as expected.

These results, in conjunction with the previously noted failure of etiocholanolone (3α-hydroxyetiocholan-17-one) to provoke fever in rabbits (1), indicate that there is a high degree of species specificity to the fever-producing action of steroid hormone metabolites. The physiologic basis of this species specificity is not clear, since the mechanism by which these steroids provoke fever in humans is not known. The phenomenon is itself of intrinsic biologic interest, however, and may imply the special significance of these or structurally related endogenous compounds to thermogenic processes under certain circumstances in man. The probable participation of steroid pyrogen in the febrile mechanism of disease has indeed already been indicated (6), and the possible role of pyrogenic progesterone metabolites in the temperature elevation of pregnancy and the luteal phase of the menstrual cycle has been suggested (3, 4).

The species specificity of steroid-induced fever is, moreover, one of a number of biologic properties which distinguish steroid from bacterial pyrogens (7). The thermogenic activity of the latter substances in susceptible animals has provided the most widely used experimental technique for investigations on the pathogenesis of fever. The endogenous origin of pyrogenic steroids, the species specificity of their thermogenic action and their availability as pure compounds through chemical synthesis, however, would seem to offer unique advantages to their use in studies on the mechanism of fever directly in man. Studies along these lines are in progress in these laboratories.

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December 8, 1959

Acknowledgments

We wish to express our thanks to Dr. Elwood V. Jensen, Department of Biochemistry and Ben May Laboratory for Cancer Research, The University of Chicago and Dr.

* Operated by the University of Chicago for the United States Atomic Energy Commission.

David K. Fukushima, The Division of Steroid Metabolism and Biochemistry, Sloan-Kettering Institute for Cancer Research, New York, for generous gifts of steroids.

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THREE SULFATE ESTERS OF 17-KETOSTEROIDS IN THE PLASMA OF NORMAL SUBJECTS AND AFTER ADMINISTRATION OF ACTH

To the Editor:

Until now, direct evidence of the existence of plasma 17-ketosteroid (17-KS) sulfates has not been available except in 1 case of adrenal tumor (1). On the other hand, Oertel and Eik-Nes (2) have isolated phosphoric acid-dehydroepiandrosterone complexes in the plasma of normal men receiving corticotropin (ACTH).

Methods have been recently developed by Migeon and Plager (3), Ceresa and Cravetto (4) and Saier and associates (5) for the measurement of plasma 17-KS. The first two technics involve an ether extraction procedure at pH 1 (6), and the third an ethyl-acetate solvolysis (7); these have been demonstrated to cleave the steroid sulfate esters. We report here the chromatographic evidence of the presence of sulfate esters of 17-KS in plasma.

METHODS AND RESULTS

Two pools of plasma were collected from blood drawn between 11 and 12 a.m. Separation of the plasma from the red cells was undertaken immediately. Plasma pool N came from the blood (1,200 ml.) of 3 normal ambulatory subjects (2 men and 1

TABLE 1. TOTAL 17-KETOSTEROID CONJUGATES AND SULFATE ESTERS IN S FRACTIONS OF PLASMA*

Plasma fractions	N_S (μg./100 ml.)	A_S (μg./100 ml.)
17-KS (Callow, Callow and Emmens (11))	57	283
Ester-SO_4^- (Roy-Vlitos-Crépy) (12, 13)	86	588

* Standard: Na-dehydroepiandrosterone sulfate.

woman) 20 to 40 years old, and pool A from the blood (350 ml.) of 4 convalescent men 20 to 45 years old who had each received two intramuscular injections of ACTH-zinc, *viz*, 40 I.U. at fifteen hours and 40 I.U. at three hours before the blood collection. Plasmas were lyophilized and successively subjected to extraction by hexane, dichloromethane and methanol in a Kumagawa apparatus; the alcoholic fractions alone were chromatographed on alumina columns. Fractions of the ethanol-acetone eluates (S fractions) containing the majority of the conjugated 17-KS were studied.

These S fractions did not contain free ketosteroid, glucuroconjugate or phosphate, as determined from paper chromatography and electrophoresis analysis followed by chemical detection methods (8–10). In Table 1 are shown the values found by Callow and Emmens' technique for 17-KS (11), and by the Roy-Vlitos-Crépy method for sulfate esters (12, 13). On a molecular basis, the excess of sulfate esters over 17-KS is evident. By subsequent paper chromatography and electrophoresis, unknown sulfates besides the 17-KS sulfates were also detected.

A portion of N_S was subjected to paper electrophoresis in borate buffer, 3×10^{-2} *M*, pH 8.9, under 6×10^{-2} $V\times m^{-1}$. A single Zimmermann positive zone was visualized, which migrated as synthetic sodium sulfate esters of dehydroepiandrosterone, androsterone and etiocholanolone. On the same zone, SO_4^{--} was detected after dioxane-HCl hydrolysis by the rhodizonate-barium chloride method (14).

Aliquots of N_S and A_S were chromatographed on paper with iso-amylic alcohol-water-ammonia (9). One spot was detected which was positive for 17-KS and SO_4^{--} and had the same $R_f\sim0.7$ as the 3 synthetic sulfates.

An aliquot of N_S was successively subjected to treatment with the enzymes of *Helix pomatia* (L) digestive juice, the HCl-ether extraction procedure, and hot acid hydrolysis. The eluate of the $R_f\sim0.7$ zone of a previous chromatogram was treated in the same way. In both experiments, the free steroids were separated by the ligroin-propanediol system (8) and measured.

An eluate of the $R_f\sim0.7$ zone of the A_S chromatogram was solvolyzed by the ethyl-acetate procedure (7) and the free steroids chromatographed in the ligroin-propanediol (8) and the cyclohexane-methanol-water (Baulieu, unpublished data) systems. Another aliquot of A_S was directly chromatographed in the system "B, 5–10" (ligroine, t-butanol, isopropyl

TABLE 2. 17-KETOSTEROID SULFATES IN S FRACTIONS OF PLASMA*

Plasma fractions	N_S (μg./100 ml.)	A_S (μg./100 ml.)
Dehydroepiandrosterone sulfate	25.5	78
Androsterone sulfate	14.5	23
Etiocholanolone sulfate	6.0	13

* Expressed as sodium salt.

oxide, ammonia-water, 2:3:5:1:9, v/v/v/v/v; Baulieu, unpublished data), which separates the sulfates of dehydroepiandrosterone, etiocholanolone and androsterone.[1] In both experiments, Zimmermann chromogens were measured.

Table 2 shows the amounts of the 3 sulfate esters directly and indirectly measured. Some unidentified polar chromogens were detected on the chromatograms; they may partly explain the difference between total (Table 1) and analytical (Table 2) values.

COMMENTS

The chromatographic identification of 17-KS sulfates involves 2 iterative stages (15) for plasma pool N, as in the case of the adrenal tumor previously reported (1). Firstly, adsorption and partition chromatography and electrophoresis yield evidence for sulfo-esterification; secondly, identification of the 17-KS is obtained by partition chromatography after hydrolysis. The total procedure is made specific by the succession of qualitatively different stages, the use of the solvolysis procedure, and the improved chromatographic technics for the separation of the free 17-KS (especially for discrimination of etiocholanolone from epiandrosterone, and of androsterone from 3β-etiocholanolone).

In addition, direct separation of the 3 sulfates is obtained for the plasma pool A_S in the system "B, 5–10," which constitutes a one-step procedure to provide a detailed picture of the 17-KS sulfates.

Nevertheless other physicochemical determinations, such as infrared spectrophotometry, are lacking for the free and conjugated steroids. Therefore they must be considered as only tentatively identified.

The existence of the 3 sulfates in blood plasma is also plausible on the basis of the capacity for sulfo-esterification of the three 17-KS in human subjects. Dehydroepiandrosterone sulfate has been found in urine of normal males (16), in adrenal tumors (1, 17) and after administration of the steroid (18); androsterone sulfate has been found after administration of testosterone (18) or androsterone (Baulieu, unpublished data), and in a

[1] No Zimmermann chromogens of the polarity of 17-KS, glucuro- and phosphate-conjugated, were seen on this "B, 5–10" chromatogram of the A_S fraction.

case of testicular tumor (19); and etiocholanolone sulfate has been detected in urine after administration of the free steroid (Baulieu, unpublished data).

The plasma values in the present study are at the lower limit of the range published until now for dehydroepiandrosterone and androsterone (3, 20). After administration of ACTH, dehydroepiandrosterone increases relatively more than the two other steroids.

Our method involving lyophilization, methanolic extraction and chromatographic isolation of conjugates has permitted the detection in blood of tentatively identified etiocholanolone as a sulfate, a finding which had not yet been described in the literature (21) except as the occurrence of the free compound in special pathologic cases (22). The plasma levels are lower than those of sulfo-conjugated androsterone; this relationship also holds true for the urine, according to our experiences with the selective hydrolytic procedure (23).

In some fractions of the alumina chromatogram of the plasma after administration of ACTH, appreciable quantities of androsterone and etiocholanolone glucuronidates were detected, using a modified Schneider system 6 (18). No phosphate of 17-KS was observed; but, when using pure K_2-phosphate-dehydroepiandrosterone, it could not be recovered from alumina columns. A total picture of the plasma 17-ketosteroids involves several types of chromatography and/or hydro(solvo)lysis, before measurement.

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Acknowledgments

We wish to thank Prof. M. F. Jayle for permanent help and advice; the U.C.L.A.F. (Prof. Velluz and Mr. Poirier) for the gift of steroids; Mr. R. Zelnick and Mr. R. Emile-ozzi for giving pure synthetic 17-KS sulfates; Dr. G. W. Oertel for a gift of K_2-phosphate-dehydroepiandrosterone; and Misses M. de Vigan and C. Corpechot for technical help.

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The Endocrine Society

The 1960 Annual Meeting

The Forty-Second Annual Meeting of The Endocrine Society will be held in the Eden Roc Hotel, Miami Beach, Florida, Thursday, Friday and Saturday, *June 9, 10 and 11, 1960.*

The Chairman of Local Arrangements is Dr. Kenneth Savard.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 10, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Eden Roc will hold 250 bedrooms and the adjoining hotels, the Montmartre Hotel and Lucerne Hotel, will hold 75 rooms each for members until May 1, 1960, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 12 for the American Medical Association and other meetings, it is imperative that your reservation be made from June 12 on, directly through the A.M.A. Housing Bureau, c/o Miami Beach Convention Bureau, 1700 Washington Avenue, Miami Beach, Florida, since no reservation beyond June 12 can be accepted directly by the hotels. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1960.

Final program, membership card and advance registration forms will be sent on May 1, 1960 to members whose current dues have been paid.

(For program, see the April issue of the Journal)

The 1960 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

The Ciba Award

The Ciba Award, to recognize the meritorious accomplishments of an investigator in the field of clinical or preclinical endocrinology who has not attained his thirty-sixth birthday by June 1 of the year in which the award is presented, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Rob-

erts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer; 1959—Dr. Gordon L. Farrell. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of

The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

Twelfth Postgraduate Assembly in Endocrinology and Metabolism

UNDER THE CO-SPONSORSHIP OF THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

MADISON, WISCONSIN

October 24 through 28, 1960

For further information, please write to the local chairman, Dr. Edgar S. Gordon, The University Hospitals, 1300 University Avenue, Madison 6, Wisconsin. The fee is $100.00 for physicians and $30.00 for Residents or Fellows taking the course.

First International Congress of Endocrinology

The First International Congress of Endocrinology will be held in *Copenhagen, Denmark, July 18–23, 1960.*

United States members of the International Executive Committee are Dr. Warren O. Nelson and Dr. Leo T. Samuels, Dr. Gregory Pincus is Chairman of the Program Committee. The tentative program consists of ten symposia covering the general field of endocrinology plus round-table discussions and a number of submitted papers. Those desiring to submit papers should immediately contact Dr. Gregory Pincus, Worcester Foundation, Shrewsbury, Massachusetts. There will be a number of interesting scientific and technical exhibits.

In order to facilitate travel arrangements to the Copenhagen Convention, the Council of The Endocrine Society at its San Francisco meeting designated THOMAS COOK & SON as our authorized travel agents. They will in turn offer especially designed All Expense Paid Tours to Europe, including hotel reservations for the convention, as well as offering independent itineraries for any of our members who desire to travel separately by air or by steamship.

Registration is open to anyone interested in endocrinology at an approximate fee of $28 U.S. Members of the families of those attending may

register as associates at an approximate fee of $14 U.S., fee to be paid at the time of registration or in advance to the American Express Company, which has been designated by the Executive Committee of the International Congress as official financial and travel agent. There will be a number of social functions and other entertainment provided for all registrants.

As you all know, European travel is unusually heavy during July and it is suggested that those contemplating attending the Congress should start making tentative reservations at the earliest possible moment by filling in and returning the card recently mailed to members, to THOMAS COOK & SON's special representative—Harry E. Kornbaum, Transportation Chairman, Rainbow Travel Service, 2817 Classen Boulevard, Oklahoma City 6, Oklahoma, who will correspond directly with each member about his individual travel arrangements to and from the Convention, at the same time keeping our National Officers fully informed of all travel arrangements of its members. Completion of the card CARRIES NO OBLIGATION OR COMMITMENT ON YOUR PART. Send it in today for the complete travel arrangements for our Society.

The general expenses of a meeting of this magnitude are quite large and The Endocrine Society has made a substantial contribution from its treasury for organizational purposes. U. S. pharmaceutical firms have also contributed very generously. However, sufficient funds are not as yet available to assure the success of this meeting; therefore, *the committee suggests that each of our members make voluntary contributions to this fund.* Please make your checks payable to: The Endocrine Society—First International Congress of Endocrinology. Mark it *Contribution* and mail it to the office of the Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

Program

The program of the First International Congress of Endocrinology which will be held in Copenhagen, Denmark, July 18–23, 1960, will consist of ten symposia and a round table discussion, along with sessions of communicated papers. The speakers at the symposia and the round table discussion have been invited by the Subcommittee on Program of the Congress. In Symposia #1–8 and in #10, the Subcommittee has also invited a discusser for each principal paper. Symposium #9 will consist of six principal papers and no discussers. The round table discussion will consist of eight sections, each with a principal speaker and three secondary speakers.

(For program see the December 1959 issue of the Journal.)

The American Goiter Association

1960 Annual Meeting and the 4th International Goiter Conference

The next Annual Meeting of the American Goiter Association will be held in *London, England, July 5–8, 1960,* at Church House, Dean's Yard, Westminster, London, S.W.1. This meeting, co-sponsored with the London Thyroid Club, will be the Fourth International Goiter Conference. For further information write Dr. Selwyn Taylor, Royal Society of Medicine, Dering Yard, 67c New Bond Street, London, W.1, England.

JOHN C. MCCLINTOCK, *Secretary,*
149½ Washington Avenue,
Albany 10, N.Y.

Scientific Program

Tuesday Afternoon, July 5

Assembly Hall

Chairman: Sir Charles Harington

14.00	Address, The President of The American Goiter Association.	
14.30	Greer, M. A., Iino, S. and Barr, S. (USA)	The role of progoitrin in human goitrogenesis; studies on the mechanism of conversion of progoitrin to goitrin.
14.50	Allcroft, R. and Salt, F. J. (GB)	Goitrogenic factors in kale (*Brassica oleracea*).
15.10	Peltola, P. (Finland)	The goitrogenic effect of milk obtained from an endemic goitre region in Finland.
15.30	Howel Evans, W. and Kitchin, F. D. (GB)	The role of PTC taste response in thyroid disease.

Chairman: Professor Bernard Houssay

15.50	Fraser, G. R., Morgans, M. E. and Trotter, W. R. (GB)	Sporadic goitre with congenital deafness (Pendred's syndrome).
16.10	Thould, A. K. and Scowen, E. F. (GB)	The syndrome of congenital deafness and simple goitre.
16.30	Van Wyk, J. J., Wynn, J., Arnold M. B., Deiss, W. P., and Graham, J. (USA)	Genetic studies in simple goiter.
16.50	Choufoer, J. C. (The Netherlands)	Further observations on congenital hypothyroidism with defective dehalogenation of iodotyrosines.

Special Lecture Theatre

Chairman: Professor E. J. Wayne

14.30	McClintock, J. C. and Gassner, F. X. (USA)	Antithyroid drugs in the treatment of hyperthyroidism.

14.50	Wollman, S. H. and Reed, F. E. (USA)	Inhibition by thiocyanate of radioiodine uptake by the thyroid gland.
15.10	Mayberry, W. E. and Astwood, E. B. (USA)	The effect of propylthiouracil on the intrathyroid metabolism of iodine in rats.
15.30	Rimington, C. (GB)	Relation between chemical structure and antithyroid activity.

Chairman: Professor J. Roche

15.50	Maloof, F. and Soodak, M. (USA)	The transsulfuration of the sulfur of thiourea to protein (PB-S) in thyroid tissue.
16.10	Hetzel, B. S., Good, B. F., Wellby, M. L. and Charnock, J. S. (Australia)	The effect of salicylate on plasma protein-bound iodine in various states of thyroid function.
16.30	Clode, W., Sobral, J. M. and Baptista, A. M. (Portugal)	Bromine interference in iodine metabolism and its goitrogenic action.
16.50	Escobar del Rey, F. and Morreale de Escobar, C. (Spain)	Effect of thiouracil, methylthiouracil and propylthiouracil on the metabolism of thyroid hormone in thyroidectomized, l-thyroxine maintained rats.

Wednesday Morning, July 6

Assembly Hall

Chairman: Sir Charles Harington

09.20	Baranov, V. G., Blagosklonnaja, J. B., Davidowski, N. M., Loskutova, E. A. and Nikolaenko, N. F. (USSR)	The nervous factor in pathogenesis of thyrotoxicosis.
09.40	Becker, D. V., Furth, E., Nunez, E., Horwith, M., Berman, M. and Ray, B. (USA)	A detailed study of persistent thyroid function following total hypophysectomy in patients with Graves' disease.
10.00	Yamazaki, E. and Noguchi, A. (Japan)	The effect of thyroid surgery on the plasma level of BEI^{131} and turnover rate of thyroxine.
10.20	Lamberg, B.-A., Burstein, J. and Eramaa, E. (Finland)	Myocardial infarction in thyrotoxicosis.

Chairman: Professor Gilbert-Dreyfus

10.40	Osserman, K. E. and Silver, S. (USA)	The differential diagnosis of myopathy as seen in hyperthyroidism and myasthenia gravis.
11.00	Lawson, J. D. and Weissbein, A. S. (USA)	The Achilles reflex contraction time as an aid in evaluating adequacy of treatment in hypothyroid patients.
11.20	Skanse, B. (Sweden)	The difference in serum cholesterol between pituitary and primary hypothyroidism.
11.40	Catz, B. and Russel, S. (USA)	Myxedema with shock and coma; a report of 5 survival cases.
12.05	The Dunhill Memorial Lecture.	

Special-Lecture Theatre

Chairman: Professor A. Vannotti

09.20	Alexander, D., Buchanan, W. W., Crooks, J., Wayne, E. J., Anderson, J. R. and Goudie, R. B. (GB)	The association of thyrotoxicosis and Hashimoto's disease.
09.40	McConahey, W. M., Keating, F. R., Butt, H. R. and Woolner, L. B. (USA)	Comparison of laboratory tests for the diagnosis of Hashimoto's thyroiditis.
10.00	Pulvertaft, R. J. V., Doniach, D., Roitt, I. M. and Hudson, R. V. (GB)	Cytotoxic effects of Hashimoto serum.
10.20	Trotter, W. R. and Belyavin, G. (GB)	Complement-fixation reactions between a thyroid antigen and serum from patients with thyroid disease.

Chairman: Professor C. Cassano

10.40	Vickery, A. L. and Hamlin, E. (USA)	Struma lymphomatosa; observations on repeated biopsies.
11.00	Milcu, S.-M., Lupulescu, A., Pop, A. and Simionescu, L. (Rumania)	Recherches immunologiques sur le goitre experimental et sur les maladies de la thyroide humaine.
11.20	Irvine, W. J. (GB)	An investigation of the pathogenesis of Hashimoto's disease (lymphadenoid goitre) by thyroid tissue culture.
11.40	Hellwig, C. A. (USA)	Experimentally induced thyroiditis.

Wednesday Afternoon, July 6

Assembly Hall

Chairman: Professor J. H. Means

14.00	Bartels, E. C. and Ire, M. (USA)	Thyroid function in patients with progressive exophthalmos requiring orbital decompression.
14.20	Milcu, S.-M., and Augustin, M. (Rumania)	Experimental researches on endocrine exophthalmos.
14.40	Dobyns, B. M. (USA)	Assay of exophthalmos-producing substance (EPS) in patients with exophthalmos.
15.00	Condliffe, P. G., Bates, R. W. and Albert, A. (USA)	Evidence for the separateness of pituitary thyrotropin and the exophthalmogenic factor.

Chairman: Dr. Rulon W. Rawson

15.20	Purves, H. D. and Adams, D. D. (New Zealand)	An abnormal thyroid stimulator in the sera of hyperthyroid patients.
15.40	Halmi, N. S., Granner, B. A., Doughman, D. J., Peters, B. H. and Müller, G. (USA)	Biphasic effect of TSH on thyroidal iodide collection in rats.
16.00	Rosenberg, I. N., Athans, J. C., Behar, A. and Ahn, C. S. (USA)	Thyrotropin-induced release of iodide from the thyroid.

16.20	El Kabir, D. J. (GB)	The estimation of thyrotropic hormone in blood by a sensitive *in vitro* assay method.
16.40	Mess, B. (Hungary)	Effect of lesion of habenular nuclei on pituitary TSH content and blood level.

Special Lecture Theatre

Chairman: Professor S. Lissitzky

14.00	Lindsay, S. and Jenks, P. R. (USA)	Enzyme histochemistry of the thyroid gland.
14.20	Nadler, N. J., Carneiro, J. and Leblond, C. P. (Canada)	The rate of synthesis, secretion and breakdown of thyroglobulin in the mouse thyroid follicle.
14.40	Bates, R. W. and Warren, L. (USA)	Studies on the sialic acid content of thyroid glands.
15.00	Vilkki, P. (USA)	*In vitro* studies on phospholipid metabolism of the thyroid.

Chairman: Professor Stephan Milcu

15.20	Lewitus, Z., Anbar, M. and Gutman, S. (Israel)	Contribution to the understanding of the "trapping" mechanism in the thyroid gland.
15.40	Dumont, J. E. (Belgium)	Carbohydrate metabolism in thyroid tissue *in vitro*.
16.00	DeGroot, L. J. (USA)	The early phase of hormonogenesis in thyroid tissue.
16.20	Sos, J., Kemeny, T., Kertai, P. and Rigo, J. (Hungary)	Tyrosine metabolism and the thyroid gland.
16.40	Lissitzky, S., Roques, M. and Benevent, M-T. (France)	Tyrosine-deiodinase: purification, properties and physiological signification.

Thursday Morning, July 7

Assembly Hall

Chairman: Professor V. G. Baranov

09.30	Bastenie, P. A., De Visscher, M., Beckers, C., Ermans, A., Vandenschrieck, H. G. and Galperin, H. (Belgium)	Endemic goitre in the Uele region (Belgian Congo).
09.50	Falkiewicz, A., Pacynski, A. and Ruzyllo, E. (Poland)	Die Kropfkarte in Polen.
10.10	Szabó, G., Balogh, I. R. and Demeczky, M. (Hungary)	Untersuchungen zur endemischen Kropffrage auf Grund der Bodenkunds.
10.30	Kilpatrick, R., Broadhead, G. D., Edmonds, C. J., Munro, D. S. and Wilson, G. M. (GB)	Studies on goitre in the Sheffield region (England).
10.50	Sigurjonsson, J. (Iceland)	The small iodine-rich thyroid.

Chairman: Dr. S. F. Haynes

11.10	Perinetti, H., Barbeito, A., Gangiani, R. J., Giner, M., Parisii, M. and Paturzo, E. O. (Argentina)	The use of iodized salt in a region of endemic goitre.

11.30	Costa, A., Fregola, G. and Marocco, F. (Italy)	Comparisons between endemic goitre, cretinism, deaf-mutism and sporadic goitre, cretinism, deaf-mutism.
11.50	Koenig, M. P. (Switzerland)	Studies of thyroid function in endemic cretins.
12.10	Kicic, M., Miltinovic, P., Djordjevic, S. and Ramzin, S. (Yugoslavia)	Endocrinological aspects of an endemic of cretinism.

Special Lecture Theatre

Chairman: Professor K. Fellinger

09.30	Cassano, C., Baschieri, L. and Andreani, D. (Italy)	A study of some cases of simple goitre with a high renal clearance of iodine.
09.50	Dimitriadou, A., Fraser, R., Slater, J. D. H. and Wagner, H. (GB)	Abnormalities of iodine transfer within the thyroid gland in non-toxic goitre.
10.10	Feldman, J. D. (USA)	Metabolism and fine structure of the iodine deficient thyroid.
10.30	Mason, S. and Farran, H. E. A. (GB)	Iodide retention in the human thyroid.

Chairman: Professor A. Querido

10.50	Baschieri, L., Andreani, D., de Luca, F., Negri, M., Salabe, G. B., Pinchera, A. and Menzinger, G. (Italy)	The metabolism of labelled *l*-thyroxine in hyperthyroidism, in myxedema and in normal subjects.
11.10	Aleshin, B. V. (USSR)	The significance of the nervous system in the response of the thyroid to iodine deficiency.
11.30	Baschieri, I., Pavoni, P. and Semprehene, L. (Italy)	"Curved" scintigraphy of the thyroid.
11.50	Fellinger, K., Höfer, R. and Vetter, H. (Austria)	Clinical and laboratory observations of patients with hyperactive thyroid nodules.
12.10	Liuzzi, A., Slingerland, D. W. and Burrows, B. A. (USA)	Thyroidal release of non-thyroxine iodine in hyperthyroidism.

Friday Morning, July 8

Assembly Hall

Chairman: Professor M. Saegesser

09.30	Walthard, B. (Switzerland)	Der Einfluss der Jodprophylaxe des Kropfes auf die Häufigkeit und Struktur der Struma maligna.
09.50	Percy, N. M., Seed, L., Milles, G. and Kearns, J. E. (USA)	Cancer of the thyroid; an evaluation of patients from three institutions.
10.10	Winship, T. and Rosvoll, R. V. (USA)	Childhood thyroid cancer; data on 515 patients, 100 followed from 10 to 33 years.
10.30	Thomas, C. G. (USA)	Studies on the dependency of thyroid cancer.
10.50	Ackerman, N. B., Shahon, D. B. and Marvin, J. F. (USA)	A new method for the diagnosis of thyroid cancer; the radioactive phosphorus uptake test.

Chairman: Dr. H. Wijnbladh

11.10	Woolner, L. B., Beahrs, O. H., Black, B. M. and McConahey, W. M. (USA)	Classification and behaviour of thyroidal carcinoma; a review of 30 years' experience.
11.30	Frantz, V. K. and Yannopoulos, K. (USA)	Carcinoma of the thyroid; a clinicopathologic study of 225 cases with ten-year follow-up.
11.50.	Money, W. L. and Rawson, R. W. (USA)	The effect of *l*-thyroxine and 3,5,3-triiodothyropropionic acid on the induction of dibenzanthracene tumors in the rat.
12.10	Pochin, E. E. (GB)	The occurrence of leukaemia following radioiodine therapy.

Special Lecture Theatre

Chairman: Professor P. A. Bastenie

09.30	Starr, P. (USA)	Observations on the metabolic and clinical effects of sodium dextrothyroxine medication.
09.50	Lerman, J. (USA)	Dissociation of response of myxedema to "triac": comparison with small doses of thyroid.
10.10	Ruegamer, W. R. and Silverman, F. R. (USA)	3,5 Diiodothyroacetic acid and the control of tissue cholesterol and lipid concentration in the hypercholesterolemic rat.
10.30	Green, W. L. and Ingbar, S. H.(USA)	Studies of the metabolism of tetra- and triiodothyroacetic acids in man.

Chairman: Dr. George Crile

10.50	Hellman, L., Bradlow, L., Zumoff, B., Fukushima, D. K. and Gallagher, T. F. (USA)	The influence of thyroid on steroid metabolism; a "thyromimetic" effect of of androsterone.
11.10	Degrossi, O. J., Houssay, A. B. and Varela, J. E. (Argentina)	Erythrokynetic studies in anaemia of thyroidal and pituitary insufficiency.
11.30	Gasiorowski, W. (Poland)	Pyruvic acid level, glutamic oxalacetic transaminase (GOT) and aldolase activity in blood in hyperthyroidism.
11.50	Cizkova, G. (Czechoslovakia)	Thyroid hormones as differentiating factors.
12.10	Galton, V. A. and Ingbar, S. H. (USA)	Thyroid hormones in the frog and tadpole; the relation of hormonal degradation to hormonal effect.

Friday Afternoon, July 8

Assembly Hall

Chairman: *Sir Geoffrey Keynes*

14.00	The Van Meter Prize Essay.	
14.15	Vannotti, A. and Béraud, Th. (Switzerland)	Distribution of iodine compounds in the serum in thyroid disease.

14.35	Klein, E. (Germany)	Der periphere Thyroxinumsatz bei sporadischem Kretinismus mit und ohne Hypothyreose.
14.55	Mitchell, M. L., O'Rourke, M. E. and Harden, A. B. (USA)	Alterations in serum thyroxine binding demonstrated by resin adsorption.

Chairman: Dr. H. D. Purves

15.15	Blumberg, B. S. and Robbins, J. (USA)	Thyroxine-serum protein complexes.
15.35	Andreoli, M., de Luca, F., Pinchera, A. and Andreani, D. (Italy)	The plasma protein binding of hormones in hyperthyroidism; conventional and continuous flow electrophoresis and chromatography.
15.55	Hamolsky, M. W., Ravin, H. A. and Freedberg, A. S. (USA)	Further studies on the plasma protein-thyroid hormone complex in man.
16.15	Milcu, S.-M. and Niculescu-Zinca, D. (Rumania)	La liaison extrathyroidienne proteique de l'iode peut constituer un facteur determinant de la fonction thyroidienne.
16.35	Dowling, J. T., Hutchinson, D. R., Hindle, W. H. and Kleeman, C. R. (USA)	Studies of iodine metabolism during normal sub-human, primate and human pregnancy.

Special Lecture Theatre

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14.15	Taurog, A. (USA)	Extrathyroidal thyroxine formation in completely thyroidectomized rats.
14.35	Lein, A. and Dowben, R. M. (USA)	The utilization of thyroxine and triiodothyronine by isolated rat diaphragm.
14.55	Florsheim, W. H. and Velcoff, S. M. (USA)	A study of the effect of 2,4-dichlorophenoxy acetic acid on the function of rat pituitary-thyroid system.
15.15	Roche, J., Michel, O. and Closon, J. (France)	Le metabolisme de l'ester sulfurique de la 3:5:3-triiodo-L-thyronine (ST_3) chez le rat.

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15.35	Bach, I., Braun, S., Gáti, T., Kertai, P., Sos, J., and Udvardy, A. (Hungary) P., Sos, J. and Udvardy, A. (Hungary)	Die Wirkung des Rubidiums auf der Schilddrüse.
15.55	Feuer, G. (GB)	Variations in thyroid hormones after injecting thyrotropic hormone in normal and selectively bred rats.
15.55	Greif, R. L. (USA)	The effect of a "thyroxine inhibitor" upon the swelling of isolated rat liver mitochondria.
16.15	Saka, M. O. and Imren, H. (Turkey)	The effect of oxidized fatty acids on the respiratory activity of liver homogenate from thyroidectomized and hyperthyroid rats.

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Book Register

Biochemistry of Human Genetics. Ciba Foundation Symposium edited by G. E. W. Wolstenholme, O. B. E. and C. M. O'Connor, B.Sc. This symposium was initiated by Professor Montalenti of the Institute of Genetics, University of Naples, on behalf of his own department and the International Union of Biological Sciences. Twenty-nine participants from all parts of the world discussed: Genetical Analysis in Man, Biochemical Genetics as Illustrated by Hereditary Galactosemia, Cholinesterase Types, Genetical Variation and Sense Perception, The Genetics of Primaquine Sensitivity of the Erythrocytes, Genetics of the Plasma Protein Variants, Hereditary Gamma Globulin Groups in Man, The Approach by Tissue Culture, and The Mechanism of Gene Action; 348 pages; 60 illustrations; 1959. Little, Brown and Company, Boston, Mass. Price $9.50.

Blood Pressure and Subarctic Climate in the Soviet Union. Survey of the Russian Literature and Investigations on Delayed Repatriates. By Dr. Bruno Hoffmann. Medical Series No. 16 of the Reports of the Osteuropa-Institute of the Berlin Free University, published by Prof. Dr. Max Brandt. English translation made available by Dr. E. A. White, Research Associate, I.C.R.S., Fordham University. A compilation of data on the climatico-meteorologic susceptibility of blood pressure in the human organism based on the blood pressure records of 1203 World War II German prisoners of war. The National Institutes of Health, Bethesda, Maryland, through the Russian Scientific Translation Program will distribute this publication at no charge to cooperating libraries.

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No. 4. Virus Virulence and Pathogenicity. In honor of Prof. J. Mulder, Department of Internal Medicine, Medical School, University of Leiden. Edited by G. E. W. Wolstenholme, O.B.E. and C. M. O'Connor, B.Sc. for the Ciba Foundation. Seventeen participants discussed

The Definition and Measurement of Virus Virulence, Host-Cell Factors and Virus Virulence, The Effect on Virulence of Changes in Parasite and Host, Broad Aspects of the Problem of Human Virulence in Influenza Viruses, The Severity of Influenza as a Reciprocal of Host Susceptibility, and The Virulence for Man of Some Respiratory Viruses Passed in Tissue Cultures; 114 pages; 13 illustrations; 1960. Little, Brown and Company, Boston, Mass. Price $2.50.

Explorations in the Physiology of Emotions. Psychiatric Research Report No. 12 of the American Psychiatric Association, edited by LOUIS JOLYON WEST, M.D., Professor and Head, Department of Psychiatry, Neurology and Behavioral Sciences, University of Oklahoma School of Medicine, Oklahoma City, and MILTON GREENBLATT, M.D., Assistant Superintendent and Director of Research, Massachusetts Mental Health Center; Associate Clinical Professor of Psychiatry, Harvard University Medical School. Scientific papers and discussions of a Regional Research Conference held April 19 and 20, 1957 at the University of Oklahoma Medical Center. Discussions, reviews, and summaries of many years of work are presented with a view to calling attention to the physiology of the emotions; 272 pages (paper); illustrated; 1960. American Psychiatric Association, 1700 Eighteenth Street, N.W., Washington 9, D.C. Price $2.00.

Klinische Physiologie. Aktuelle Probleme in Übersichten. Volume I. Part 1. By Dr. W. A. MÜLLER, Chefarzt der II. Inneren Abteilung des Robert-Bosch-Krankenhauses, Stuttgart. (In German.) The various chapters cover the fields of the primary action of ionizing rays in tissues, the basic phenomena of the EEG, sugar transport in cells and tissues, and metabolic-genetic abnormalities; 118 pages (paper); 38 figures; 1960. Georg Thieme Verlag, Stuttgart, Germany; Intercontinental Medical Book Corporation, New York 16, N.Y. Price $5.50.

La Fatigue. Physiologie, psychologie et médecine sociale. By PIERRE BUGARD, Docteur en Médicine, Docteur ès-Sciences, Chef de Laboratoire Adjoint du Centre d'Endocrinologie de l'Hôpital Laënnec, Paris. Introduction by PROF. DR. ALBEAUX-FERNET. (In French.) The importance of the problem of fatigue demands study as a whole, as attempted in this book which correlates chapters on the effects of stress, nervous and muscular factors, the autonomic nervous system, psychosomatic aspects, the adrenal cortex, and the various clinical pictures as noted in medical practice, sports, industry and aviation. Other chapters are devoted to aids in diagnosis, the prevention of fatigue, and the curative treatment; 308 pages (paper); 33 figures; 1960. Masson et Cie, Éditeurs, Paris 6, France. Price, 32,00 NF.

Les Oedèmes, Physiopathologie et traitement de la rétention de sel et d'eau. By JEAN FABRE, Privat-Docent à la Faculté de Médecine de Genève. Preface by PROF. R. S. MACH. (In French.) In the first part a review is given of the most recent contributions to the metabolism of salt and water, including partition of electrolytes, hormonal and renal regulation, and the maintenance of fluid volume in the body. The second part is devoted to an analysis of the mechanisms of edema and their relationship to disorders of the heart, kidney, liver and endocrine glands. The third part deals with the treatment of edema, particularly the use of the new diuretics and adrenal hormones; 344 pages (paper); 46 figures; 1959. Masson et Cie, Éditeurs, Paris 6, France. Price 55,00 NF.

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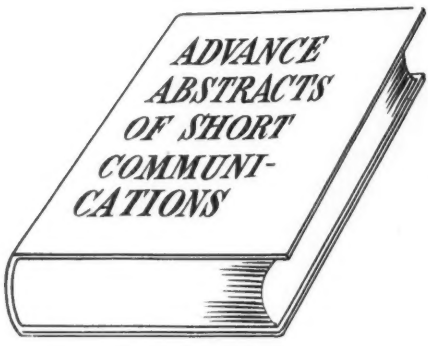

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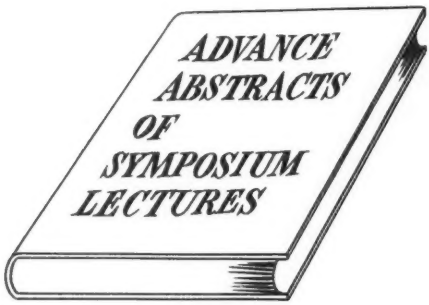

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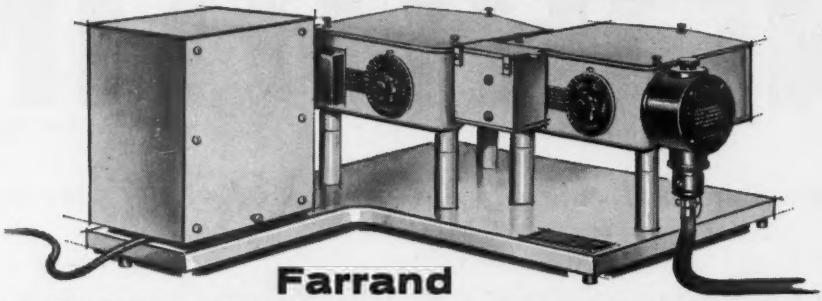

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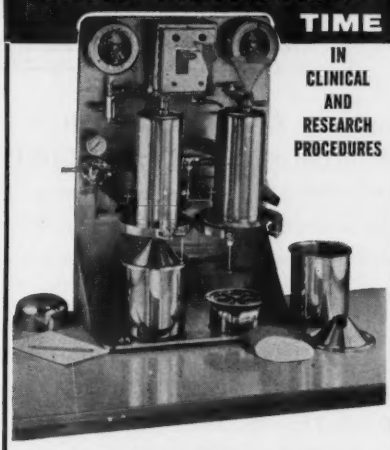

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